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THE TREATMENT OF DYSFUNCTIONAL UTERINE BLEEDING WITH TESTOSTERONE PROPIONATE

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WITH THE RECENT availability of the chemically pure and highly concentrated androgens, the medical profession is confronted with the problems of therapeutic indications, margin of safety and optimal dosage. Therapeutic indications and margin of safety of a new therapeutic agent are primarily suggested by the response of animal organs to different quantities of the product. An analysis of the current literature on the effects of testosterone propionate in female animals permits the following conclusions.

A. Short treatment (1 to 10 days) produces indifferent or stimulative changes in the ovary of mature and immature rats, depending largely on the dose employed (1, 2, 3).

B. Prolonged treatment (62 to 102 days) produces definite degenerative changes in the rat ovary. This effect is, however, temporary, inasmuch as there is restitution of function and partial restitution of structure in the adult rat within 29 days after withdrawal of treatment (4). The ovary of the mature rhesus monkey reacts similarly to prolonged testosterone treatment (5).

C. Whether the period of treatment is short or long, testosterone propionate inhibits the gonadotropic function of the anterior pituitary lobe of the rat and prevents the occurrence of castration changes therein (6, 7). The initial ovarian stimulation incident to short treatment despite pituitary inhibition may, as suggested by Hamilton and Wolfe (6), be caused by a temporary release of accumulated pituitary sex hormone or by a direct stimulation of the ovary (8).

D. Whether the period of treatment is short or long, testosterone propionate invariably causes actual or relative uterine growth in the intact and castrated rat (9, 4, 10) indicating a direct stimulative action on the Muellerian tract like that of estrogen. Short treatment also produces a progestin-like effect on the uterine mucosa of the castrated rat similar to that seen in pregnancy (9, 10, 11).

Although conclusions derived from experimental observations on animals may not be applicable to the human being, the pituitary-ovarian inhibition

resulting from prolonged treatment of the female rat with testosterone propionate nevertheless suggested its use in women with dysfunctional uterine bleeding, especially in those of menopausal age in whom an overproduction of the follicle-stimulating hormone is held to be responsible for the continuous production of estrogen and consequent endometrial hyperplasia and uterine bleeding (12).

Whether the human ovary responds to the administration of testosterone as does the animal ovary cannot be ascertained without examination of excised ovaries after a period of treatment. It is known, however, that the administration of the substance in adequate doses (450 to 1000 mg. in 30 days) limits the production of the gonadotropic hormone in the castrated woman (13), temporarily suppresses the menstrual cycle (14, 15), often arrests dysfunctional uterine bleeding (15, 16), and produces atrophic changes in the endometrium (16, 17, 18). These laboratory and clinical manifestations suggest that in the human female also large doses of testosterone given over a period of a month inhibit the development of ovarian follicles and estrogen production through suppression of anterior pituitary lobe function.

The smaller doses (30 to 120 mg. in 30 days) employed in most of the cases herein reported were, however, almost equally effective in arresting abnormal uterine bleeding without disturbing the menstrual rhythm of those who menstruated regularly but bled excessively and without changing the normal endometrial pattern in the 3 patients who were curetted both before and after the period of treatment, suggesting that in these patients testosterone propionate acted directly on the unknown bleeding factor of the uterus and not through the mediation of the pituitary-ovarian mechanism.

CLINICAL OBSERVATIONS

Selection of cases. The material comprising this study consists of 38 women, 25 premenopausal and 13 younger women, ranging in age from 22 to 51 with an average of 40 years. Twenty-nine of the 38 patients had metrorrhagia for periods averaging 12 weeks with a minimum of 4 weeks. Nine had menorrhagia for an average period of 17 months with a minimum of 4 months. Thirty of the patients menstruated regularly prior to the inception of the abnormal bleeding, 7 had delayed periods, and 1 had a radium-induced menopause 10 months previously. Only 3 of the 38 patients had evidence of small fibroids. In order to eliminate the possibility of a fundal malignancy as the cause of the abnormal bleeding, a preliminary curettage was performed in 23 of the older patients. Three of the 23 were also curetted at the termination of the testosterone treatment.

Therapy. The patients were treated with testosterone propionate in sesame oil[1] administered intramuscularly 3 times weekly for from 2 to 9 weeks. The individual dose varied from 2.5 to 25 mg. The total dosage per month ranged from 30 to 300 mg. The number of patients receiving each dosage level is indicated in figure 1.

[1] The authors are indebted to Drs. Gregory Stragnell and Erwin Schwenk of the Schering Corporation for the generous supply of testosterone propionate (oreton) used in these experiments.

Results. A cure was considered to have been effected if the abnormal bleeding ceased during the period of treatment and did not recur for at least 4 months after cessation of treatment. The follow-up period ranged from 4 to 19 months with an average of 8 months. Patients who were only improved are classified as 'not cured.'

By these criteria, 26 of the 38 patients (68%) were cured and 12 (32%) were only temporarily or not at all improved by the therapy. In order to determine whether the preliminary curettage significantly influenced the number of cures, the results in the curetted and non-curetted groups are

TESTOSTERONE PROPIONATE IN DYSFUNCTIONAL UTERINE BLEEDING, NUMBER OF PATIENTS, 38; AGE OF PATIENTS, 22 TO 51; MEAN, 40

TYPE AND DURATION OF ABNORMAL BLEEDING

Type	No. of Patients	DURATION	
		Minimum	Average
Metrorrhagia	29	4 weeks	12 weeks
Menorrhagia	9	4 months	17 months

DOSAGE

Dose	No. of Injections	No. of Patients
2.5 mg.	12–20	16
5 mg.	7–20	11
10 mg.	7–28	7
25 mg.	10–20	4

Results During Follow-Up Period of 4 or More Months

Total Cures:	26 of 38 (68%).
With Curettage	17 of 23 (74%).
Without Curettage	9 of 15 (60%).

compared. Of the 23 curetted prior to treatment, 17 (74%) were cured. Of the 15 who were not curetted, 9 (60%) were cured. It is seen, therefore, that while preliminary curettage apparently increased the percentage of cures, the results even in the non-curetted group are encouraging, particularly as compared with the use of the APL substance which is known to be ineffective at the menopausal age (19).

Other effects. No constitutional or local ill-effects were observed in any of the 38 patients included in this report. The menstrual rhythm in the 30 patients who were menstruating regularly prior to the onset of the abnormal uterine bleeding was not disturbed. There was no obvious tendency toward a reduction of fertility by the doses herein employed. Four of the patients conceived within 1 to 10 months after withdrawal of treatment without a tendency to abortion. Three of the patients were delivered of normal infants and one had an induced abortion. This is in accord with the experimental observation of Scipiades (20) who found that 5 to 65 mg. of testosterone did not reduce the subsequent fertility of rats. The menopausal symptoms complained of by a number of patients were not influenced by doses herein employed.

The possibility of evoking masculine characteristics with the use of large doses of testosterone over a long period of time must, however, be borne in mind. This tendency to virulism, though mild and temporary, was noted in 4 patients who were treated with large doses of testosterone for other conditions than dysfunctional uterine bleeding. In 2 of the 4 there was an apparent increase of facial hair. In the other two, girls of 14 years of age, there was a definite decrease in the pitch of the voice.

SUMMARY

From a survey of the literature and the study herein reported, it is evident that testosterone propionate has a definite place in the treatment of dysfunctional uterine bleeding, especially at the menopausal age when the APL substance is almost totally ineffective and when large doses can be employed without risk of permanent damage to the ovaries.

Larger doses than those used in this study, namely an average of 30 mg. per day for 27 days (13) cause temporary amenorrhea through inhibition of the gonadotropic function of the anterior lobe of the pituitary gland. The possible occurrence of facial hair growth and masculinization of the voice must, however, be borne in mind when large doses are employed.

Smaller doses of testosterone propionate, such as 30 to 120 mg. per month are almost equally effective without, however, disturbing the menstrual rhythm or causing atrophy of the endometrium, suggesting that the action of the substance in this dosage is probably a direct uterine effect and not through inhibition of the anterior pituitary lobe.

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HIBERNATION AND THE ENDOCRINES[1]

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IN THE ANIMAL KINGDOM adverse environmental conditions are met by many interesting adaptations: certain protozoa develop cysts; many insects pupate; the Australian lungfish burrows into the mud and secretes a gelatinous capsule which enables it to survive the dry season; certain mammals in the temperate zone react by an interesting and complex state known as hibernation. These mammals depend upon insects and grasses for their chief source of food. Many of them live in frozen subterranean burrows during the long winter months. Characteristically they are able to tolerate cold (Horvath's Test),[2] they have an unstable temperature regulating mechanism, they show a striking increase in their body weight during the summer months and are sexually inactive during the winter. This phenomenon of hibernation is a seasonal defense reaction in which homiothermic forms become poikilothermic, thus enabling them to survive long periods of low temperature and diminished food supply at the expense of their extensive fat deposits.

The physiological characteristics of hibernating animals are profoundly changed from those of the non-hibernating state. The body temperature is only slightly higher than freezing. Nervous irritability is depressed to such a degree that surgical procedures may be executed without anesthesia. The respiratory rate is less than 5 per minute during deep hibernation and there is a marked reduction in O_2 consumption with retention of carbon dioxide, the venous blood containing approximately 80 volumes % (1, 7). There is also a bradycardia, hemoconcentration and marked reduction in the peripheral circulation (2).

The phenomenon of hibernation has challenged the interest of investigators since the time of Spallanzani (3). Claude Bernard (4) observed that the livers of animals which hibernate have a high glycogen content before hibernation takes place. DuBois, (5) calling attention to the relative hemoconcentration with a high carbon dioxide content of the blood, advanced the 'autonarcosis theory' of hibernation. Since his time numerous theories have been suggested (reviews: 2, 6, 7). In 1915 Cushing and Goetsch (8) studied the histological changes of the endocrine glands in hibernating woodchucks and concluded that hibernation resulted from a wave of polyglandular inactivity. Later, Dr. Cushing was impressed by the similarity of the symptoms observed in hypophysectomized dogs and hibernating animals such as the tendency to obesity, reduction of general activity, impairment of peripheral circulation and hypo-

[1] Aided by a grant from the Wisconsin Alumni Research Foundation. The authors wish to acknowledge the advice and criticism of Dr. F. L. Hisaw in the pursuit of this problem.

[2] Hibernating forms will survive a body temperature of 19° C.; non-hibernators will not survive.

tension. Since that time many investigators have studied the various physiological factors in relation to hibernation, but little has been done in regard to actual endocrine relationships involved. This paper is presented as further evidence that the endocrine glands have a definite rôle in the process of hibernation.

METHOD

These studies have been made on thirteen-lined ground squirrels, *Citellus tridecemlineatus* (Mitchill). This form is a monocyclic rodent which in its natural habitat emerges from the winter burrow about April 1st. The males emerge 1 to 3 weeks before the females and though emaciated, sexual development proceeds at a rapid rate with descent of the testes and spermatogenesis within 3 to 4 weeks. The females undergo a similar period of rapid sexual development which results in swelling of the vagina, canalization and mating usually about May 1st. Pregnancy lasts 27 days and lactation about 6 weeks. Shortly after lactation, complete gonadal involution occurs in both sexes (9, 10, 11). The animals were taken from their natural environment during the summer and fall seasons and allowed to remain in the laboratory until they were ready to be used. In preparation for hibernation they were placed in individual cages containing nesting material, dry food and no water. Although certain groups of animals were given fruit and water this was not the routine procedure. After the animals were weighed they were placed in a cold room where the temperature was kept at 36 to 40° F. Studies were made upon normal animals during the different seasons both with and without water. In addition, a study was made regarding the effect of glandular hyperactivity upon the tendency to hibernate during the non-breeding season. This was effected by the administration of extracts from the pituitary, thyroid, adrenal and gonads. By contrast, the extirpation of these glands was carried out during the breeding season when the animals were resistant to cold and could not be induced to hibernate.

The results were tabulated to show the percentage relationship between the total number of days the animals were in the cold room and the number of days they actually hibernated. In most instances sluggishness was noted within 24 to 28 hours and actual hibernation in 48 to 72 hours. It is therefore evident that 9 to 10 days actual hibernation out of a 12-day period of exposure (75 to 80%) would be the maximal period of hibernation to be expected.

Seasonal Variations as Related to the Reproductive Cycle

In order to study seasonal variations of the tendency to hibernate, groups of 15 to 20 animals of both sexes were put into the cold at monthly intervals for periods of 12 to 14 days. The results are shown in graphic form (fig. 1). In October, a season when males were inactive sexually, there was an average of 11 days actual hibernation out of a possible 14, or 74% of the entire time. At the time of the descent of the testes in January this decreased to 55%, and after descent of the testes when the scrotum was enlarged and pigmented they were able to resist the cold without hibernating. This resistant state persisted until the middle of August, several weeks after complete gonadal regression, when the testes were abdominal and the accessories had undergone complete atrophy. At this time there was an average of 80% days hibernation.

The results in the females were similar. In the months of August to January there was consistent hibernation for approximately 70% of the time the

animals were in the cold. There was little change in this tendency to hibernate during the gradual transition from anestrum to proestrum, but with the appearance of vaginal swelling in the latter part of February, there was a fall in the number of days hibernation to 35%. Among the groups of estrous and pregnant females tested during May and June hibernation occurred very rarely. The same resistant state persisted throughout the period of lactation, and as in the males was terminated at about the time of complete gonadal regression.

These results show that hibernation can be induced much easier in sexu-

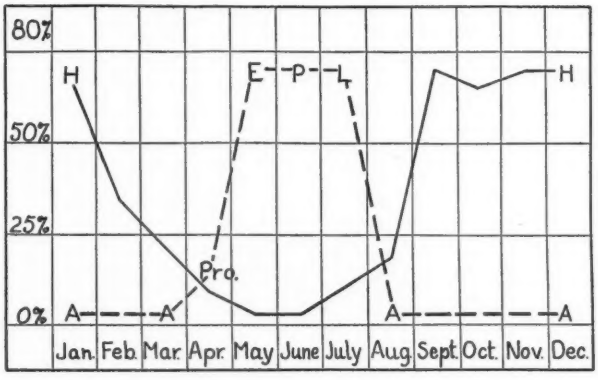

Fig. 1. Seasonal variations in the tendency to hibernate as related to the reproductive cycle. H-Graph represents percentages of days actual hibernation for groups of 10 to 20 animals exposed to the cold 10 to 16 days at monthly intervals. The reproductive cycle is represented by the broken line: A, Anestrum; Pro, Proestrum; E, Estrus; P, Pregnancy; and L, Lactation. The reproductive cycle in the male and the tendency to hibernate parallels the above very closely.

ally inactive animals. As sexual activity supervenes, the percentage of hibernation is reduced progressively until hibernation cannot be induced during the breeding season.

Effect of Dehydration

Based on a 6-year period of observation involving hundreds of squirrels exposed to hibernating temperatures it seemed inescapable that experimental hibernation was subject to numerous unexplainable variations. For example sexually inactive males frequently exhibited a remarkable ability to resist the cold. In an attempt to determine the factor responsible for this variation it was observed that those animals having an inadequate water supply hibernated more consistently than did those having fluids. Therefore a series of 32 males was studied to determine the effects of dehydration. This experiment was carried out in September, a season when hibernation could be induced quite readily in females, but less consistently in males. Eighteen animals were kept without water for one day before being put into the cold room, where only dry food was given to them. During a period of 14 days

this group hibernated 74% of the time and lost 14% body weight. The control group of 14 males was given water before and during the period in the cold. They hibernated 12% of the time with an average weight loss of 46%. The increased weight loss may be explained in part by the increased activity of the control group. Similar results were obtained throughout the hibernating season. This demonstrates that dehydration which probably exists in the natural environment plays a definite rôle in the induction of hibernation. Other workers have shown that dehyration caused a profound disturbance in the heat regulating mechanism of higher mammals (12).

Hibernation and the Pituitary

A series of sexually active animals, both male and female, was hypophysectomized. Atrophy of the gonads and other secondary endocrine organs resulted, but the ability to resist cold was retained for 7 to 10 days. After this time, however, there was a marked sensitivity to cold with animals developing the hibernating state more quickly than under normal conditions. Hibernation lasted only 3 days and unless the animals were removed from the cold, death ensued. Due to the depletion of glycogen reserves in hypophysectomized animals it is probable that hypoglycemia was a factor in their death (13).

Attempts to convert sexually inactive (poikilothermic) animals into homiothermic forms by means of pituitary extracts were successful. A preliminary treatment with thyrotropic preparations[3] over a 3-day period followed by dehydration and exposure to cold resulted in a period of activity lasting 7 to 10 days as contrasted with the controls which hibernated during this period. The thyroid glands of the treated animals were increased from 6 to 30 mg. in weight and were hyperplastic. However, in view of recent reports concerning the direct metabolic function of the pituitary (14) it was considered advisable to determine the effectiveness of these extracts upon thyroidectomized animals. This was done in the same manner with 3 groups of 3 animals each, during the months of November and December. The treated animals remained awake 7 to 10 days and were very pugnacious while controls were hibernating throughout the entire period of exposure to cold. Collip's metabolic hormone preparation failed to maintain this 'cold-resistant state' for more than three days, but its period of activity seems to be of brief duration in other forms (14).

Therefore, it can be stated that the removal of the pituitary gland increased the incidence of hibernation converting homiothermic mammals into a poikilothermic state when exposed to the cold. The opposite reaction can be elicited during the non-breeding season by administering pituitary extracts.

Effect of Thyroid

Several investigators have studied the effects of thyroid on hibernating mammals. Adler (15), using a subcutaneous injection of thyroid extract in

[3] The thyrotropic preparations used in these experiments were prepared according to the method of Greep. Others were derived from commercial growth hormone preparations (antuitrin-G and polyansin.)

hedge-hogs, was able to produce an awakening, but his findings were questioned by Zondek (16) who demonstrated that warm saline solution would also cause waking. Johnson (17) and his co-workers were unable to confirm Adler's findings by the use of thyroxin both intraperitoneally and orally in ground squirrels. No studies have been reported upon the effects of thyroidectomy on hibernation during the active breeding season when animals are resistant to cold. Our approach to this problem was based upon the ability of these animals to resist the cold during their breeding season at which time the thyroid is hyperactive histologically as contrasted with their susceptibility to cold during the non-breeding season when the thyroid is histologically inactive.

Five animals were thyroidectomized during the early breeding season and their response to cold tested at monthly intervals. These animals could not be induced to hibernate, showing no alteration in their reaction to cold from those of the control or normal animals. In none of these was there noticeable testicular atrophy as reported by others nor evidence of residual thyroid tissue to histological examination (18).

A second group of animals was studied to determine the effect of experimentally induced thyrotoxicosis upon the seasonal susceptibility to cold. This was supplemented by studying the effects of potassium iodide and dinitrophenol upon the responsiveness to hibernating conditions.

Eight sexually inactive squirrels were fed desiccated thyroid by gavage (2 gm./kg.) either before or during hibernation and controls were given normal saline or sucrose. If the animal was hibernating, care was taken to keep the temperature of the solutions and instruments the same as that of the hibernating chamber. Those animals receiving the desiccated thyroid before being put into the cold were resistant to the hibernating state for 5 to 10 days longer than the controls. If the animals were already hibernating a period of activity resulted which lasted about 5 days. This was followed by a return to the hibernating state until a second gavage or injection was given. The group of animals fed desiccated thyroid hibernated 24% of the time in the cold room with an average weight loss of 14% as contrasted with the controls which hibernated 74% of the time with a weight loss of 14%. Cold saline or 5% glucose intraperitoneally or by gavage did not result in waking.

A similar group of animals was injected intraperitoneally with thyroxin (2½ to 4½ mg. each) over a 3-day period before being put into the hibernating chamber. Two of this group remained resistant to hibernation during the entire 14 days in the cold room, one died on the 4th day (4½ mg. dosage) and the others remained active 5 to 10 days. This group hibernated on an average of 2.5 days or 19% of the total period, as contrasted to the control group which hibernated on an average of 12 days or 73% of the time. The former lost 24% body weight and the latter 11%.

A third group of animals received thyrotropic pituitary extracts either intraperitoneally during hibernation or subcutaneously before exposure to the cold. Those receiving the intraperitoneal injection were awakened for a period of 5 to 10 days. There was an average increased weight of the thyroid

TABLE 1

EXPERIMENTAL SERIES								CONTROLS						
Date	No. animals and sex	Sexual condition	Treatments Unless otherwise stated these were administered before exposure to cold.	Results: Days in cold	Days of hibern.	% Days hibern.	Average wt. loss %	No. animals and sex	Sexual condition	Treatment	Results: Days in cold	Days of hibern.	% Days hibern.	Average wt. loss %
Oct.	18, M	In.	One day dehydration; no water in cold, dry food only	14	11	74	14	14, M	In.	Water ad lib.	12	1.5	12	46
June	3, M	Act	Hypophysectomy	6	2	33		3, M	Act	None	6	0	0	
June	4, M	Act	Hypophysectomy	10	2	20		2, M	Act	None	10	0	0	
June	4, F	Act	Hypophysectomy	13	3	23		4, F	Act	None	13	0	0	
Nov.	8, M	In.	0.4 gm. Thyrotropic daily 5 days before exposure	12	3	25	22	8, M	In.	None	12	9	74	14
Nov.	3, F	In.	to cold	13	1	8	36	3, F	In.	None	13	10	77	12
Nov.	6, M	In.	10 U Ant.-G. daily, 5 days	10	4	40	18	8, M	In.	None	12	9	74	14
Nov.	6, M	In.	10 to 50 U Ant.-G. daily, 5 days; thyroidectomy 7 da. prev.	10	1	10	22							
Nov.	5, M	In.	Thyroidea des. (2 gm./kg.) by gavage	12	2	16	20							
	3, F	In.		12	2	16	34	3, F	In.	None	12	8	67	9
Nov.	7, MF	In.	Thyroxin 2.5 to 4.5 mg. intraperitoneally in a single injn.	13	2.2	16	24			Above				
Nov.	5, M	In.	2 R.U. FSH daily, 10 days	10	4.2	42	21			Above				
Aug.	3, M	In.	Same	10	2	20		3, M	In.	None	10	6	60	
Mar.	3, F	In.	2 R.U. FSH daily, 5 days	10	1	10	36	3, F	In.	None	10	3.5	35	14
Nov.	4, M	In.	0.5 R.U. LH subcutaneously daily, 10 days	16	4	25	18			Above				
Nov.	8, M	In.	0.5 to 1.0 gm. equivalent of LH intraperitoneally during actual hibernation	15	10	67	20	8, M	In.	Saline i.p.	15	10	67	16
Nov.	6, F	In.	50 to 100 R.U. Theelin in aqueous solution intraperitoneally during actual hibernation	No Waking						Above				
Nov.	8, MF	In.	1/4 to 1 cc. Cortin injected intraperitoneally during actual hibernation	No Waking						Above				

gland from 10 to 45 mg. and an associated irritability and aggressiveness. Histologically a hyperplasia was present in the thyroid gland. When hibernation occurred following a period of induced activity there was evidence of regression in the thyroid gland. These animals hibernated 1 to 4 days or 16% of the total period in the cold room with an average weight loss of 25%. The controls hibernated 10 to 12 days with an average of 65% of the period, associated with a weight loss of 14%.

A group of 8 animals was treated with potassium iodide (½ cc. 5% soln.) intramuscularly before being put into the cold. There was a suggestion of increased susceptibility to cold with the treated group hibernating 74% of the time and the controls hibernating 64% of the period, but this difference is not considered to be of significance.

Finally, a small series of hibernating animals was treated with dinitrophenol (¼ to 1 mg.) intraperitoneally in a single injection. Waking occurred in 2 to 5 hours and was preceded by a sharp rise in respiratory and heart rates. Thus, it is demonstrated that hyperthyroidism by direct or indirect measures (desiccated thyroid, thyroxin or thyrotropic pituitary extracts) results in a transient homiothermic state for the duration of thyroid activity. However, extirpation of the thyroid does not alter seasonal homiothermism, suggesting an independent metabolic thermo-regulating function derived probably from the pituitary gland.

The Adrenals and Hibernation

The adrenal glands have long been considered to play a dominant rôle in the hibernating state (19). Not only is there atrophy of the adrenal gland during hibernation, but there are changes in the blood which are very similar to those of adrenal insufficiency: hemoconcentration, slowing of peripheral circulation, and possibly hypoglycemia. In addition, certain hibernating mammals have been shown to tolerate extirpation of the adrenals for long periods of time, particularly if they are subjected to actual hibernation (20). The above changes relate to cortical insufficiency, but the medullary portion of the adrenal may possibly play a rôle as evidenced by the fact that an intact medulla is apparently necessary for normal shivering in response to hypoglycemia which process is probably involved in awakening. This is further borne out by recent observations (unpublished) which indicate that intraperitoneal injections of adrenalin into hibernating ground squirrels will stimulate heart rate, respiratory rate and in many instances produce convulsions with rise in temperature and an awakening. This active state is transitory, however, and hibernation may be resumed within a short time.

Although these various symptoms implicate the adrenal as a factor in the process of hibernation, removal of the adrenals during the breeding season failed to alter the animals' ability to resist cold. In a group of 8 animals placed in the cold after adrenalectomy all remained awake until shortly before their death which occurred on about the 7th day. This was essentially the same survival period for adrenalectomized animals in the warm room. A similar group of animals adrenalectomized in October survived about 7 days with no

appreciable difference between those in the cold and those in the regular quarters.

Eight hibernating animals treated with cortin (½ to 1 cc.) intraperitoneally did not awaken or show an observable effect from the injection.

From these studies it appears that the adrenal does not play a dominant rôle in the phenomenon of hibernation. Although adrenalin causes awakening it is of transitory nature and apparently not related to the 'cold-resistant state' of the breeding season, as the latter is uninfluenced by adrenalectomy.

The Gonads and Hibernation

The seasonal nature of the homiothermic state in hibernating mammals and its association with the breeding season has already been emphasized. However, it has been pointed out by other workers (21) that extirpation of the gonads during the breeding season does not wholly eradicate this seasonal homiothermic state. Furthermore, the administration of estrogenic hormones has been demonstrated to have little effect on hibernation. Since castration has little effect when carried out during the breeding season, it was felt that perhaps castration had been executed too late to interfere with the pluriglandular wave of hyperactivity in which the gonads played only a minor part. Therefore, a group of 12 animals was castrated the first of January and their tendency to hibernate compared with that of normals. The castrated animals hibernated 9 days out of 12 in the cold while the normals hibernated 6 days. The results indicate that there is a moderate alteration in the seasonal homiothermism when castration is performed before the inception of that state.

The minor rôle of the gonads was further demonstrated by the administration of gonadotropic extracts[4] to sexually inactive and hypophysectomized animals after complete gonadal involution. In both circumstances the follicle stimulating fraction of the pituitary gland produced estrus in the females with enlargement of the genitalia and canalization of the vagina, and spermatogenesis in the males without enlargement of the accessories. In neither situation was there an appreciable alteration in the tendency to hibernate. These results were not completely duplicated with all extracts, but were subject to the thyrotropic content involved. Luteinizing pituitary extracts had no demonstrable effect on sexually inactive females, while males were stimulated to an increased liberation of the male sex hormone as evidenced by pigmentation of the scrotum, enlargement of the accessories and in a few instances descent of the testes without a significant increase in size or stimulation of the germinal epithelium.

Administration of 50 to 100 R.U. of theelin before the hibernating period was also without significant effect upon the tendency to hibernate. Similarly, intraperitoneal injection of theelin failed to cause an awakening in animals already hibernating.

Thus it has been possible to activate the sexual apparatus directly and

[4] The gonadotropic preparations used in these experiments were prepared according to the method of Fevold and under his supervision.

indirectly in both sexes during the inactive season and following the removal of the hypophysis without influencing the hibernation phenomenon. Hibernation has been induced in females which were anatomically speaking in full estrum, and in males with active spermatogenesis.

DISCUSSION

The seasonal nature of the hibernation phenomenon has been established, substantiating the hypothesis of Cushing and Goetsch regarding the rôle of polyglandular inactivity as an etiological factor. Further study eliminates the gonads and adrenals as major factors and implicates the pituitary and thyroid as the most important factor. Extirpation of the pituitary during the breeding season resulted in a change from the homiothermic to the poikilothermic state but such animals were unable to survive prolonged exposure to the cold even though actually hibernating. This failure to survive is probably related to the disturbance in carbohydrate and fat metabolism which has been demonstrated in other forms after hypophysectomy (22). Extirpation of the thyroid failed to alter the homiothermic state, suggesting a direct metabolic function of the pituitary. This was established by showing that pituitary extracts are capable of inducing a 'cold-resistant state' in thyroidectomized animals during the non-breeding season when they would normally hibernate very readily. Substitution therapy by thyroid or other means of producing hypermetabolism converted poikilothermic animals into homiothermic ones only for the duration of the metabolic stimulus.

SUMMARY

The thirteen-lined ground squirrel was found to hibernate more consistently under experimental conditions when dehydrated to a moderate degree. During the breeding season, hibernation could not be induced, but shortly afterward there was maximal tendency to hibernate which persisted until the onset of genital enlargement. From the time vaginal swelling appeared in the female and the testes became scrotal in the male the tendency to hibernate steadily diminished until sexual activity terminated this phenomenon. During this homiothermic or breeding season, extirpation of the pituitary converted these animals into poikilotherms, while removal of the thyroid, adrenal or gonads was without significant effect. Substitution therapy during the non-breeding (poikilothermic) season with pituitary preparations resulted in transient homiothermism. Similar results were obtained with thyroid preparations, but gonadotropic pituitary extracts, corticoadrenal extracts and sex hormones were without effect. These findings suggest inactivity of the pituitary as the primary factor responsible for the phenomenon of hibernation.

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THE TERMINATION OF PREGNANCY OF DOGS BY GONADOTROPIC ANTIHORMONE

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A NON-SPECIES-SPECIFIC antihormone which inactivates the pituitary gonadotropic hormones of many species of animals is highly useful for studies of the physiological effects of antihormones. Such a preparation, previously described (1), has produced in experimental animals the following effects: *a*), inactivation of the gonadotropic hormone of the animal from whom the serum was obtained; *b*), cessation of the sexual development of young rats, *c*), inhibition of the action in test rats of pituitary gonadotropic hormones from several species of animals including man.

Another physiological action attributable to the gonadotropic antihormone was observed in the experiments herein reported, in which abortion of pregnant dogs followed the intravenous injection of this preparation. Normal canine serum, containing no gonadotropic antihormone, did not cause abortion.

EXPERIMENTAL DATA

The antihormone serum was obtained with sterile precautions from a dog that had been injected for 3½ years with a gonadotropic extract of sheep-pituitary glands. In addition to the gonadotropic antihormone, the serum contained appreciable amounts of the antidiuretic principle but it did not contain the oxytocic principle.[2] The normal serum was obtained from a large normal male dog. Details of the experiment are given in the protocol.

The pregnant dogs were secured from the following sources: 5 were bred in the laboratory and the date of breeding was therefore known; 5 were loaned by friends who were able to supply the approximate date of the breeding; 5 were pregnant when purchased, and the later events proved that these dogs were in the last half of their pregnancies.

Thirteen pregnant dogs were injected intravenously[3] with the antihormone, and each went into labor 48 to 100 hours following the first injection

[1] Alexander Brown Coxe Fellow. The experiments were aided by funds granted by the Josiah Macy Jr. Foundation, and by the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry.

[2] These tests were performed by Dr. Alfred Z. Gilman, Laboratory of Pharmacology, Yale University School of Medicine.

[3] Two of the animals (experiments 2 and 9) first were given subcutaneous injections; the serum, which was poorly absorbed, had no effect. Intravenous injections then were followed after the proper interval by abortion.

PROTOCOL[1]

Experiment No.	Identification of dogs	Stage of pregnancy at time of labor	Treatment given	Day of treatment when labor occurred	State of fetuses
1	299	last half	*Antihormone* 5 cc. i.v., 2/d, 3½ d; Total 35 cc.	3rd and 4th days	10 dead.
2	300	last half	*Antihormone* 5 cc. s.c., 1/d, 3 d; 10 cc. s.c., 1/d, 4 d; 10 cc. i.v., 1/d, 1 d; Total 65 cc.	9th day	1 dead, 3 survived and were raised.
3	300 2nd abortion	49th day	*Antihormone* 5 cc. i.v., 2/d, 2½ d; Total 25 cc.	3rd day	4 died soon after birth.
4	301	last half	*Antihormone* 5 cc. i.v., 2/d, 2½ d; Total 25 cc.	3rd day	5 found dead.
5	301 2nd abortion	52nd day	*Antihormone* 5 cc. i.v., 2/d, 3 d; Total 30 cc.	4th day	Fetuses eaten during night.
6	316	last half	*Antihormone* 5 cc. i.v., 2/d, 3 d; Total 30 cc.	4th day	4 seen alive, 4 dead; all died prematurely.
7	306	last half	*Antihormone* 10 cc. i.v., 2/d, 2 d; Total 40 cc.	3rd day	Fetuses eaten during night.
8	*Dr. T.'s dog 1*	47th day	*Antihormone* 10 cc. i.v., 1/d, 3 d; 7 cc. i.v., 1/d, 1 d; Total 37 cc.	4½ days	Fetuses eaten during night.
9	*Dr. T.'s dog 2*	last half X-ray showed 1 fetus	*Antihormone* 5 cc. i.m., 2/d, 4 d; 10 cc. i.v., 2/d, 6 d; Total 160 cc.	10th day	1 fetus died soon after birth.
10	*Dr. C.'s dog 1*	last half	*Antihormone* 5 cc. i.v., 2/d, 2½ d; Total 25 cc.	3rd day	Fetuses eaten.
11	*Dr. C.'s dog 2*	last half	*Antihormone* 5 cc. i.v., 2/d, 2 d; Total 20 cc.	3rd day	Fetuses died, soon after delivery.
12	*Dr. C.'s dog 3*	last half	*Normal Serum* 5 cc. i.v., 2/d, 4 d; Total 80 cc.	did not abort; normal delivery 2 wk. later	2 normal pups at term.
13	*Cocker spaniel* (pathol.)	43d day	*Normal Serum* 10 cc. i.v., 2/d, 4 d; Total 80 cc. in 7 days no effect; *Antihormone* 10 cc. i.v., 2/d, 3 d; Total 60 cc.	4th day after antihormone	Fetuses eaten.
14	*Tan mongrel* (pathol.)	54th day	*Normal Serum* 10 cc. i.v., 2/d, 4 d; Total 80 cc. in 7 days no effect; *Antihormone* 10 cc. 2/d, 3 d; Total 60 cc.	4th day after antihormone	Fetuses eaten.

[1] The following abbreviations are used: i.v.—intravenously; i.m.—intramuscularly; s.c.—subcutaneously; d—day; /d—per day; cc.—cubic centimeters.

of antiserum. The labors were apparently normal. In one instance (experiment 2), however, it was suspected that the delivery was at term, because 3 of the puppies lived and were cared for by the mother; the date of expected delivery in this case was not known. With this exception, the other fetuses were premature, and the mothers showed no interest in them. Some of the fetuses were observed to be alive when they were delivered, but the mothers attempted to eat them as soon as they expired. Two of the dogs (experiments 3 and 5) were aborted twice by the method described, and a third dog has had one period of estrus following her abortion.

Two of the pregnant dogs were injected twice daily for 4 days with normal canine serum, and after 7 days during which abortion did not occur, they were injected with the antihormone which did produce abortion after the proper interval (experiments 13 and 14). A third pregnant dog was injected with similar amounts of normal serum and was not given any antihormone; 2 weeks later this animal delivered 2 normal pups (experiment 12)

DISCUSSION

Anderson and Collip (2), as may be recalled, first noted that prolonged injections of crude pituitary extract into animals induced in their sera the ability to prevent in other animals the physiological action of the pituitary extract. Collip ascribed the antagonistic action of these sera to an 'antihormone' and interpreted the phenomenon as one involving chalones (Sharpey-Shafer) rather than immune bodies. The contributions of Collip and his co-workers have been amplified by numerous other experiments which in general have tended to furnish support for the concept that the antihormone is an immune body and not an antagonistic hormone or chalone. The final solution of the perplexing problem of the identity of the antihormones necessarily awaits the purification of the hormones and antihormones.

For all practical purposes one may consider at present that the injection of a gonadotropic antiserum confers on the recipient a temporary, passive resistance (immunity) to pituitary gonadotropic hormone. Under suitable conditions an antiserum can be obtained which inactivates the pituitary gonadotropic hormones of many species of animals including dogs and man.

Votquènne reports (3) that hypophysectomy of pregnant dogs is followed by resorption or abortion of the fetuses. Rabbits also resorb or abort their fetuses if the pituitary gland is removed at any stage of pregnancy (4). On the other hand, rats, mice, and guinea pigs require the presence of the hypophysis only during the first half of pregnancy. Rowlands states that a non-species-specific anti-gonadotropic serum causes abortion of rabbits at any stage of pregnancy, but this does not occur in rats, mice, or guinea pigs, when injections are started during the latter half of pregnancy (5).

A possible criticism of most antihormone experiments can be found in a neglect or inability to eliminate a foreign protein reaction as the cause of the observed effects. The experiment reported in this communication would appear to escape this criticism because canine serum was the source of the antihormone and dogs were the subjects of the experiments.

The invariable occurrence of the abortion of the animals during the 2nd, 3rd, or 4th day following the first injection suggested the possibility that the reaction was caused by some physiological readjustment rather than by a true oxytocic principle which may have been contained in the antiserum. Tests of the serum with the guinea pig uterus failed to reveal an oxytocic effect. Further study of the properties of the antiserum showed the presence of considerable antidiuretic hormone, as has already been stated.

It seems safe to conclude that the pregnancies of the dogs were terminated by a factor present in the antihormone canine serum but not present in normal canine serum. In the active serum presumably there was sufficient antihormone specific for canine pituitary gonadotropic hormone to inactivate this pituitary factor. It has been shown that the hypophysis is necessary for continuation of all stages of pregnancy of dogs. The passive resistance (immunity) to the dog's own pituitary hormones induced by the injection may have served as a temporary physiological hypophysectomy. The delay of 48 to 100 hours between the time of the first injection and the onset of labor provides a reasonable time interval for the occurrence of these physiological processes. It is impossible, while lacking the highly purified antihormone, to state with certainty that the abortion was produced by the antihormone, but there seems little doubt that such was the case.

The antihormone apparently produced no permanent harmful effects on the mothers, and, as far as could be determined, it exerted no ill effects directly on the fetuses.

SUMMARY

The interruption of 12 pregnancies of dogs has been accomplished by the intravenous injection of a non-species-specific anti-gonadotropic canine serum.

The action of the antiserum was comparable to a physiological hypophysectomy, and it may have occurred by conferring on the injected dog a passive resistance (immunity) to her own pituitary gonadotropic hormone. Normal canine serum did not produce this effect.

The antihormone did not cause any objective permanent ill effects in 3 aborted dogs, 2 of which later became pregnant and were again aborted, and one which had a period of estrus.

The aborted fetuses which were observed at delivery were alive, but subsequently died of prematurity and lack of maternal care.

The antihormone preparation used had no appreciable oxytocic activity when tested on the guinea pig uterus, but it contained a considerable amount of antidiuretic hormone, in addition to thyrotropic and gonadotropic antihormones.

The writer acknowledges his debt of gratitude to Dr. Harvey Cushing for the encouragement and guidance so generously given during the many months of these experiments.

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THE ASSAY OF GONADOTROPIC EXTRACTS IN THE POST-PARTUM RABBIT[1]

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SEVERAL YEARS AGO the post-partum rabbit was recommended for the assay of gonadotropic materials because of the great regularity of response and the rapidity with which the results could be obtained (1). Since this suggestion was made there has appeared only one report (2) on this procedure. Most of the work on rabbits has involved the use of isolated females, erroneously designated as 'estrous.' From the very extensive work of Hill, Parkes, and White (3) it is apparent that the response of the isolated female rabbit is no more regular than that of the immature rat, so that equally large numbers of test animals must be employed. Hill, Parkes and White felt obliged to use 20 rabbits at each dose level for their standardization curves and recommend no less than 10 'estrous' animals at the critical doses in routine assays. Insofar as the use of the rabbit eliminates many of the recognized variables encountered in bioassay, one is obliged to consider that the remaining variables are relatively more important. These remaining variables in the opinion of the above authors (3) are "inherent individual variation in sensitivity, body weight, and previous treatment." Of these, only individual sensitivity cannot be determined before the assay is conducted.

The sensitivity of an animal to gonadotropic injections should be dependent upon the ovarian condition at the time of injection. It has been quite generally assumed that female rabbits may be designated as estrous after an isolation period of 30 days or more; that is, any rabbit at the end of such period will have mature follicles in the ovary, will show mating behavior when placed with a male, and will be capable of ovulating in response to the normal physiological stimulus (coitus). From the data of this laboratory and of the Cambridge laboratory (4, 5) it is clear that there is no justification whatsoever for this generally accepted assumption. The average incidence of estrus, as defined above, is not more than 50% in healthy, isolated, mature females, and shows a pronounced seasonal variation from 25% in the autumn to about 75% in the spring. Unfortunately, neither vaginal color nor any other external appearance can be relied upon for the detection of estrus (4). Inasmuch as the amount of gonadotropic material required to ovulate anestrous females (those which refuse the buck, and with no corpora lutea present at the time) may be as great as 4 to 5 times the dose required for animals in

[1] This work was supported by a grant from The Rockefeller Foundation.

heat (6), it is to be expected that the ovulating dose for any group of isolated females will show wide variarions.

The post-partum animal was selected for our assays to eliminate the uncertainties encountered in the isolated female and to insure uniform ovarian condition in all of the test animals. It has been reported by Hammond (5) and confirmed by Friedman (4) that estrus occurs with great regularity in post-partum animals and lasts for a period of at least 25 days. With such control of individual variation it is possible to detect and evaluate some of the other factors influencing the response to gonadotropic extracts. It is for this purpose that we now present the data in this report as a representative part of the results accumulated in this laboratory over a period of years. These data indicate that gonadotropic extracts may be assayed quickly and accurately with very few post-partum rabbits.

METHODS

Selection of animals for assay. To save time and space most of the animals are delivered to the laboratory when about two weeks pregnant. The feeding of a constant diet in pellet form[2] permits adequate detection of deviations in food consumption. Each animal is inspected daily and notation made of food consumption, time of littering, number and condition of the young, and the behavior of the mother toward the new-born. Almost every pregnant rabbit shows some degree of anorexia 24 to 48 hours preceding parturition. In some of the females, however, anorexia sets in during the last half or third of pregnancy. Despite the fact that these animals do not appear to be ill, they usually suffer marked abnormalities of parturition. Of those afflicted, 10 to 15% die during or shortly before labor. The survivors have abnormally long labors which result in the casting of a few young, usually macerated, over a period of 24 to 72 hours. If the uterus has been completely emptied, the anorexia disappears, and the rabbit shows no signs of ill health (until the next pregnancy when the process is repeated). In many instances, however, the uterus is not completely emptied at labor, and there are retained in utero one or more fetuses which undergo necrosis, resulting in a large, pus distended uterus. Such a uterus can be palpated and diagnosed in the living animal, but a more certain guide to the condition is the persistence of anorexia after parturition. Breeding trials have amply demonstrated that estrus does not occur with regularity either in females recovering from the protracted labors, or, in which there is uterine retention. Indeed, most of such animals are persistently anestrous, and cannot be relied upon for assay work.

For assay at the critical levels, therefore, we have used only those females which have completed a normal pregnancy, which have given birth to a litter of 4 or more healthy young, and which show a normal post-partum food consumption. Such females may be used at any time from 2 to 25 days post-partum. The other post-partum rabbits, which do not meet these strict requirements, may be used for range finding. Similar use may be made of females 20 or more days following a sterile mating (which would make available for range finding any females bred at the rabbitry but found to be not pregnant upon arrival at the laboratory). The incidence of estrus in such animals is almost as high as in the post-partum female (4).

The injection of the gonadotropic materials. The pregnancy urine extracts were stored as dry powders.[3] For each series of injections sufficient powder was accurately

[2] The composition of these pellets and the general care and management of the colony has already been described (4).

[3] We are indebted to Dr. Oliver Kamm of Parke Davis for the supply of the P. U. extracts, one of which assayed 1600 u per milligram.

weighed and made up in saline solution. The mare serum product was furnished in tablet form, 3 in each sterile ampoule, each tablet representing 5 U according to the manufacturer's assay.[4] For each series of injections a number of such ampoules were used to make up the solution in order to minimize any variation from ampoule to ampoule. The solid contents were emptied into a small volume of distilled water, and the ampoules rinsed 5 times with water in order to deliver completely any dust which may have broken away from the tablets. The anterior pituitary extracts were produced from rabbit pituitaries which had been stored in acetone. These were prepared for assay by two 1-hour aqueous extractions at a PH of about 8.0, and were injected within 30 minutes after completion of the extraction.

TABLE 1. THE INFLUENCE OF BODY WEIGHT ON THE RESPONSE OF THE POST-PARTUM RABBIT TO GONADOTROPIC MATERIAL

Material	Dose	Group	Weight Range	Response		Unit
Pregnant mares' serum	1.5	*A*	3.92 to 4.60	7	2	<0.34
		B	2.66 to 3.85	17	1	<0.39
	1.2	*C*	3.23 to 4.07	0	8	>0.37
		D	2.55 to 3.00	4	0	<0.40
	1.0	*E*	2.73 to 4.45	1	16	>0.35
		F	1.78 to 2.65	8	0	<0.38
Pregnancy urine	73	*G*	2.70 to 4.60	16	1	<16
	53	*H*	1.95 to 2.82	5	0	<18
	37	*I*	3.26 to 3.95	0	10	>11
		J	2.60 to 2.90	2	2	±13

For mare serum the dose is expressed in rat units as labelled by the maker; for P.U. it is in thousandths of a mg. of the dry powder. In both cases the amount stated represents the total amount injected. Weight range refers to the extremes of body weight of the individual rabbits in each group. The unit was calculated from the total dose and the extreme body weights as explained in the text.

All solutions for assay were made to such volume that the critical doses were contained in 3.0 to 5.0 cc. of solution. The solutions were injected into groups of 8 to 10 animals at a time. Subsequent checks on the same material were made later with solutions freshly made from the same dry product. To rule out chance variations in sampling or weighing, each series of injections was arranged to contain critical positive and negative responses. The injections were made intravenously from a syringe graduated in fifths of a cc. At laparotomy 18 to 24 hours later a count was made of the number of fresh corpora lutea, corpora hemorrhagica and cystic follicles. The presence of one or more corpora lutea was interpreted as a positive response. In the absence of fresh corpora lutea a negative response was recorded, regardless of the presence or absence of corpora hemorrhagica cystic follicles, or both.

RESULTS

The influence of body weight upon the response to a constant amount of gonadotropic extract. In table 1 are shown the results obtained at the critical dose levels with a preparation of mare serum extract and with a preparation of human pregnancy urine. At dose levels higher and lower than those in

[4] This product was generously supplied by Dr. George Cartland, Upjohn Co. The unit appears to be about 8 times as large as the usual unit for such products; i.e., each tablet being the equivalent of 40 U of most workers.

this table the responses were without exception positive and negative respectively. If the factor of body weight were not considered one would get the impression of completely irregular results, positive and negative reactions occurring at all dose levels. When weight is not a limiting factor (compare groups *B* and *C*, *D* and *E*, *H* and *I*) it may be seen that a relatively small

TABLE 2. THE BODY WEIGHTS OF THE INDIVIDUAL RABBITS OF GROUPS *E* AND *F* RESPONDING POSITIVELY AND NEGATIVELY TO A TOTAL DOSE OF 1.0 R.U. OF MARE SERUM

+	−	−
1.78	2.73	3.27
2.09	2.81	3.56
2.10	2.86	3.64
2.16	2.86	3.65
2.20	2.87	3.70
2.20	2.89	4.16
2.59	3.00	4.45
2.65	3.14	
2.83	3.25	

change in the total dose is associated with a marked change in the reaction. The influence of body weight upon the response is more strikingly illustrated by table 2 in which are listed the individual body weights and reactions of the animals in groups *E* and *F* from table 1.

If one assumes that there is a direct relation between dose and body weight, one may limit the unit (theoretical level of 50% ovulation) by the heaviest animals ovulating and the lightest animals failing to ovulate at any

TABLE 3. THE RESPONSE OF POST-PARTUM RABBITS TO EXTRACT OF MARE SERUM WHEN DOSE IS CALCULATED ON THE BASIS OF BODY WEIGHT

Dose per kg.	+	−
0.41 to 0.49	20	1
0.38 to 0.40	10	1
0.35 to 0.37	1	8
0.28 to 0.34	0	13

total dose. The resulting figure is practically the same whether it is calculated from animals weighing as little as 1.8 or as much as 3.8 kg. (see under unit, table 1). Indeed, with the exception of data from animals weighing over 4 kg. all of the data are consistent with the assumption of a linear relation between effective dose and body weight. This may be seen from table 3. The rabbits are the same as those listed in table 1, but for each animal the total dose has been divided by the weight in kilograms. The range of dosage is about 30% (from about 0.45 to 0.30 per kg.), the response above and below these levels being uniformly positive and negative respectively.

From a theoretical standpoint a linear relation between dose and body weight might not have been expected, and there is reason to believe that a straight line does not truly represent the situation. For example, a calcula-

tion of the unit from group A (table 1) would yield a figure less than 0.34 per kg., 4 positive responses having been secured in rabbits weighing from 4.04 to 4.48 kg. The deviation of this figure from the others in table 1 is of the same magnitude as the error involved in the assumption of a linear relation between energy metabolism and body weight for rabbits of 2 and 4 kg. Nevertheless, for body weights up to 4.0 kg. the data are close enough to a straight line to justify the assumption of a linear relationship for purposes of bioassay.

The sharp end point shown in table 3 is typical for assays on extracts from pregnant mares' serum and human pregnancy urine, so that it is difficult in practise to find a level giving a 50% response. One of the few examples of a divided response is shown by group *J*, table 1. With crude extracts of rabbit pituitaries, however, assayed on 4 animals at each level, a 30% reduction of the dose per kg. of body weight led to a divided response in about one-third of the assays. This indication of a flatter response curve for crude anterior lobe extracts is in harmony with the results obtained by Hill, Parkes and White (3).

Alterations in the character of the ovarian response in relation to the size of the dose. Injections of gonadotropic extracts at a level 50% or more below the unit level generally produce no detectable ovarian change. At doses slightly (20 to 30%) below the unit the ovaries commonly present several greatly distended, clear follicles of a diameter exceeding 2.5 mm, or, equally large, follicles filled with fresh blood (cystic follicles and corpora hemorrhagica respectively).

In the region of unit dose, the response is by definition qualitatively similar to the ovarian changes provoked by the normal physiological stimulus. It differs from the normal in that the number of ovulation points is obviously below the normal range in about one-half of the instances. This may be seen readily from a comparison of the ovarian response to coitus and to approximately one unit of gonadotropic extract in a group of 182 assay animals. The number of ovulation points per animal in response to the extract was 4 or less in about 40% of the observations, frequently being as low as 1 or 2. Within a period of 6 weeks post-operatively 160 of these 182 females were mated and the number of fresh corpora lutea again recorded. The average was 10.2 with a standard deviation of 2.5. Less than 5% of these animals presented as few as 6 corpora lutea, and only one presented less than six (5).

When the dose is increased to twice the unit the ovarian response is indistinguishable from the post-coital response. For example, 18 rabbits were injected with twice the unit dose of the mare serum extract. All but one of these animals revealed 8 or more rupture points at laparotomy, and this one presented 6 ovulation points. Consequently, the subliminal alterations at doses slightly below the unit, and the subnormal ovulatory response at the unit level may be used as guides in the assay procedure. It should be noted, however, that the finding of a completely normal ovarian response (8 to 12 corpora lutea) does not afford evidence against the proximity of the unit level, since about 50% of the animals injected with the unit dose respond in this manner.

Zone effects. Up to the present time a relatively crude pregnancy urine preparation has afforded us the only example of a zone effect; that is, a negative response level with a dose higher than unit. This preparation, which assayed only 150 R.U. per mg., gave the expected results with 1 to 75 times the unit dose. At 100 times the unit dose only 3 of 6 animals ovulated, and of these, none showed more than 2 rupture points at laparotomy. It should be mentioned here that this preparation was obviously toxic at this dose level. With better preparations of pregnancy urine and with the mare serum

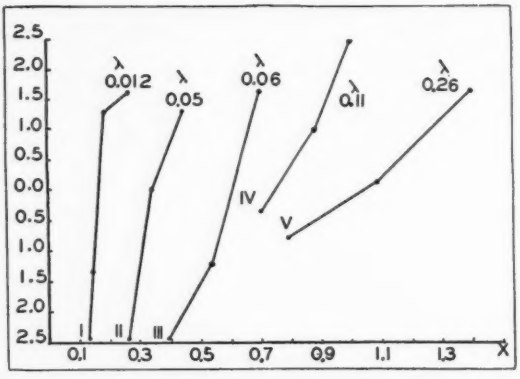

Fig. 1. THE NORMAL EQUIVALENT DEVIATION OF RESPONSE PLOTTED AGAINST THE LOGARITHM OF THE DOSE (x). For the first 3 curves the dose is per kg. body weight; for the last two, it is the total amount injected. The curves have been shifted in horizontal position for convenience. I, mare serum extract, post-partum rabbit; II, crude, P.U. extract, p.p. rabbit; III, calculated average curve for crude extract of rabbit pituitary, on p. p. rabbit; IV, P.U. extract, isolated rabbits, Hill, Parkes and White, March–April; V, P.U. extract, isolated rabbits, Hill, Parkes and White, Jan.–Feb. assay.

extracts so far tested, no zone effects have been encountered with doses from 1 to 250 times the unit.

The absence of seasonal variations in sensitivity. Due to the apparent seasonal variation in the rabbit pituitary (7) it was feared that there might also be a seasonal variation in ovarian sensitivity. Consequently, small numbers of post-partum rabbits were injected with critical doses of mare serum extract at frequent intervals throughout a 14-month period. During this time definitive assays were obtained in every month except August and September. There was no evidence of any seasonal variation in ovarian sensitivity. This finding of uniform sensitivity cannot be transferred to the isolated animal since the latter shows a marked seasonal variation in the incidence of estrus. Indeed the shifts in the slope of the curves obtained by Hill, Parkes and White are entirely in accord with what we would expect from the seasonal influence on isolated rabbits. The steep slope of their curves obtained in the spring and early summer suggests a greater uniformity of the test animals than was indicated by the slope of their curve for the winter assay (fig. 1, Curves IV, V).

Alterations in sensitivity with repeated use of the same animal. After their use in one assay, many of the rabbits were bred and used for a second assay after dropping the second litter. Such animals appear to be fully as sensitive and as reliable as fresh, post-partum animals. For range finding purposes we have often used females 3 weeks after an ovulation induced by injection, at which time the end of the induced pseudo-pregnancy should have been followed by another estrus. Although the second response in such animals gives no evidence of a marked decrease in sensitivity, we do not have sufficient data at the critical levels to recommend their use at definitive dose levels. Our data are sufficient, however, to indicate that one cannot profitably use

TABLE 4. THE STANDARD ERRORS OF GONADOTROPIC ASSAYS

Extract	Test Animal	No. per Dose Level	Standard Error
Pregnancy urine	isolated rabbit	10	25%
Pregnancy urine, crude	post-part. rabbit	2	20%
Mare serum & purified P.U.	post-part. rabbit	2	10%
Ant. pit. Crude	post-part. rabbit	2	30%
Ant. pit. Crude	post-part. rabbit	3	10%

The error for each procedure was calculated by referring to the dose-response curve the variation expected from small samples of a 50% population (by binominal expansion). The calculation for the isolated rabbit rests upon the data furnished by the paper of Hill, Parkes and White.

rabbits which have been employed for assay on two previous occasions, regardless of intervening pregnancies. Their sensitivity is obviously decreased as is witnessed by the reactions of 5 females to the injection of mare serum extract. Each of these females had been injected on two previous occasions with 1 U of mare serum extract, and each had had in the meantime two normal pregnancies. Nevertheless, the response of these animals to a third dose of mare serum was uniformly negative, although this same dose was sufficient to induce ovulation regularly in fresh post-partum females, injected at the same time.

The number of animals required for reliable results. In the preceding sections it was seen that the exclusive use of post-partum females greatly reduced the group variability in response, and that adequate provision for the influence of body weight eliminated to a very great extent the variability from animal to animal. Even with such provisions, however, there remains some variability in the response. The number of animals required, therefore, will depend upon the degree of accuracy demanded. If the unit were defined as the amount of material producing exactly 50% response, the injection of unit dose into several groups of test animals would not always produce such result. The variations from the true (50%) response would be determined by the inevitable chance variations of small samples. It is possible to calculate the extent of such variation for any size sample, and by reference to the dose-response curve translate the resulting figure in terms of per cent deviation from the true unit. By accepting as a standard error those limits of deviation which would include 70 to 80% of all assays, it is possible to compare the errors involved in various procedures. Table 4 shows such comparison.

It will be recalled that the dose reponse curve of the post-partum animal is so steep that the level of uniform ovulation (over 90%) is separated from the level of practically no ovulation by a very small difference in dose (per kg. body weight). For gonadotropic preparations other than crude extracts of anterior pituitary this difference in dose is less than 30%. Consequently, if one post-partum female were injected at each of a few critical dose levels, the positive and negative responses would define the potency 9 times out of 10 with an error less than 30%. The injection of a second animal at the level of the first negative response would constitute 2 animals at approximately the unit level, and should yield the degree of accuracy noted in table 4. A negative response in this second animal would necessitate the location of the unit by extrapolation between the positive and negative levels. For many types of investigation such determination would be entirely adequate. It more than equals the accuracy obtainable by the use of ten isolated animals at each dose. From the statistical considerations noted in the appendix of this paper, each post-partum rabbit may be expected to afford as much information as 9 to 36 isolated females.

SUMMARY

The exclusive use of post-partum rabbits for gonadotropic assays largely eliminates individual variation in sensitivity and permits an evaluation of the importance of body weight. For rabbits weighing 2 to 4 kg. a linear relation between body weight and effective dose may be accepted for practical purposes. Data from rabbits weighing more than 4 kg. suggest that this relationship is not really a straight line.

With one post-partum rabbit injected at each critical dose level (per kg. body weight) it is possible to define the biological activity of good preparations of pregnant mares' serum and human pregnancy urine 9 times out of 10 with an error less than 30%. The use of a second animal at the critical level provides an accuracy which surpasses that obtainable by the use of 10 isolated rabbits at each dose level. The dose-response curve for crude anterior lobe extracts is apparently less steep, so that more test animals are required for the same degree of accuracy. The characteristic changes at doses slightly below the unit, and the subnormal ovulatory response at the unit level may be used as guides in the assay procedure. Zone effects may be encountered with very large doses of a crude extract. They have not been seen with the use of purified extracts.

If there is a seasonal variation in the sensitivity of the post-partum animal, it is within the limits of error of the assay procedure described in this report.

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APPENDIX

STATISTICAL CONSIDERATIONS

The true levels of response as compared with the observed values. From the data presented in the preceding sections of this paper, the critical level of uniform response appears to be 95% ovulation. Since the number of observations is small (approximately 50) it is possible that the true level is either somewhat higher or lower than 95%. Even if one includes the results from 3 other assays performed concurrently on similar material (but not tabulated in this paper) there is a likelihood of about 1 in 25 (0.04) that the true value is as low as 88%. This figure was obtained by determining the probability of obtaining the actually observed values in this series of assays if the succession of samples had been drawn from a population of 88% ovulation and 12% no ovulation. Likewise, there is a likelihood of about 1 in 20 (0.05) that the samples which appear to represent 2% ovulation could have been drawn from a population in which the true percentage of ovulation was as high as 5%. It should be noted that the observed value of 2% was influenced by the one positive in 51 observations, and this was encountered at a level 10% below the level of uniform ovulation (table 3 shows this one instance). It would therefore be prudent to say that the critical levels of positive and negative response probably represent levels of 90% or more and 5% or less.

Similarly, it may be calculated that a 30% reduction in the dose of crude AP extract is probably accompanied by a change from 90% ovulation to about 12% ovulation inasmuch as such reduction in dose leads to a divided response in about one-third of 4 animal samples. A dose response curve based upon this probability was used to compute the errors listed in table 4.

The relative value of the post-partum and the isolated rabbit. One valuable method of comparing the accuracy of technic and the uniformity of the test animals is that recommended by Gaddum (8) in which the logarithm of the dose is plotted against the normal equivalent deviation of the response. When this is done the slope of the curve is related to the uniformity of the test animals and the standard deviation of the logarithm of the dose can be read directly from the graph. Figure 1 shows such curves for several assays on the post-partum rabbit and for 2 representative assays of Hill, Parkes, and White. The standard deviation of the log dose (λ) for our assays on mare serum extract and on good preparations of pregnancy urine extract is well under 0.025. The average λ for the assays of Hill, Parkes and White on P.U. extract is about 0.15 (3, p. 352). If a comparison of these two errors were justifiable, it might be said that one post-partum female should afford as much information as 36 isolated females $[(0.15/0.025)^2]$. The English workers, however, used a preparation of pregnancy urine which may not have been any better than the one cited in table 1 (curve 2, fig. 1). Even with post-partum rabbits the λ for this preparation was about 0.05. If the influence of impurity of extract is as great in the isolated rabbit assay as it apparently is in the post-partum assay, each post-partum female would have no more statistical significance than 9 solated rabbits $[(0.15/0.05)^2]$.

SEASONAL VARIATIONS IN THE GONADOTROPIC HORMONE CONTENT OF THE RABBIT PITUITARY

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IN RECENT YEARS we have become more familiar with the seasonal expression of environmental influences on reproduction. Studies on birds and mammals with obviously seasonal breeding habits have provided effective illustrations of such influences (1, 2, 3, 4, 5). But we are apt to dismiss environmental factors from consideration in those species, such as the rabbit, which breed throughout the year. Nevertheless, an inspection of actual breeding records in laboratory colonies has revealed (6) a definite seasonal variation in the incidence of estrus even in the isolated rabbit. In contrast to the isolated rabbit, the post-partum rabbit breeds with regularity at all times of the year. Consequently, we should not have expected any distinct seasonal variation in the pituitaries of post-partum females which were proven to be in estrus by actual mating. The findings reported in this paper came as an unwelcome surprise in the course of some other investigations. They are of such magnitude as to warrant special consideration.

METHODS

The donors of the pituitary glands used in these experiments were all disease-free, post-partum animals which had delivered a normal litter of healthy young, and which were consuming normal quantities of the stock diet daily (about 175 gm. of dry feed). Groups of 3 to 8 females were killed in estrus, and at definite intervals after sterile coitus (i.e., during pseudopregnancy). The animals were injected intravenously with an anesthetic dose of nembutal to permit inspection of the ovaries, after which they were exsanguinated from the aorta. The pituitary gland was then immediately removed in one piece, weighed, and dropped into 20 volumes of acetone. After being stored in the refrigerator for 2 to 5 days, the pooled glands from each group were air-dried, ground to a powder in a heavy-walled centrifuge tube and then extracted. The extraction was performed by adding to the ground glands 8 cc. of water for each gland and enough dilute NaOH to make the mixture very faintly alkaline to phenolphthalein. This alkalinity was maintained by the addition of more alkali if necessary, and the mixture stirred frequently. At the end of an hour, the tubes were centrifuged, the supernatant fluid separated and neutralized, and the residue again extracted in an identical manner for another hour. After centrifuging, the second supernatant fluid was combined with the first, 2 cc. of 10% NaCl added for each gland, and the final volume adjusted to 20 cc. per gland by the addition of distilled water.

The extracts were immediately assayed on post-partum rabbits by a method which has recently been described in detail (7). For the first series of injections 2

animals were employed at each dose level, the dose being calculated per kg. body weight of the assay animal (0.5 cc./kg., 0.66 cc./kg. etc.). The definitive doses were revealed at laparotomy 15 to 18 hours later. On the basis of these results a second series of injections was started at once, so that all injections of these extracts were completed within 24 hours after the glands had been extracted. The second series of injections was made in order to check what appeared to be the definitive doses of the first series, so that for each assay 3 to 5 animals were used at each critical dose level. During the interval between injections the extracts were stored in the refrigerator.

In a majority of the assays the unit was located by interpolation between the

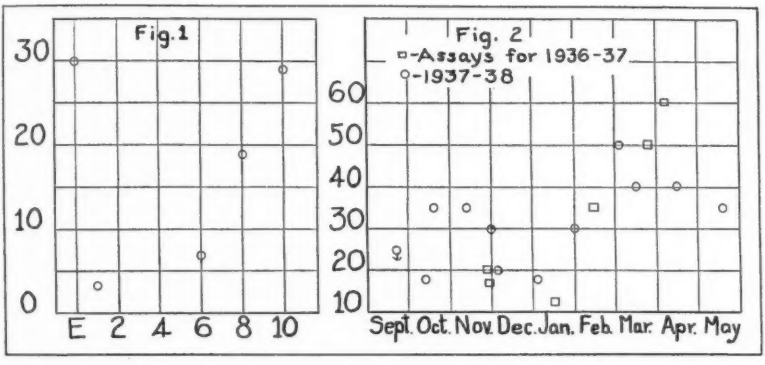

Fig. 1. THE GONADOTROPIC CONTENT OF RABBIT PITUITARIES FROM FEMALES IN ESTRUS AND IN SEVERAL STAGES OF PSEUDOPREGNANCY (from the experiments of November 1936). Fig. 2. THE GONADOTROPIC CONTENT OF RABBIT PITUITARIES AT THE 8TH DAY OF PSEUDOPREGNANCY. The assay of Oct. 13th, 1937 is subject to the criticism noted in table 1.

critical dose producing ovulation in all of the test animals and the critical dose producing ovulation in none of the test animals. (This level of uniformly negative response was never less than two-thirds of the critical positive dose level; that is about 30% below the level of all positive responses.) In the remaining assays we encountered a dose level at which positive and negative responses were about equally divided. This level was then accepted as the unit. In either case the hormonal content per gland was obtained by dividing into 20 (20 cc. per gland) the volume of extract which represented 1 U (amount required to ovulate 1 kg. of post-partum rabbit).

RESULTS

As Hill (8) has shown, the gonadotropic content of the rabbit pituitary drops sharply in the 24 hours following coitus. During the ensuing pseudopregnancy the restitution of the gland follows a characteristic curve. This curve as determined in our laboratory for the month of November 1936 is shown in figure 1. Each point on the curve is an average of the assays made on two extracts, each of which was prepared from the pooled pituitary glands of 4 to 8 rabbits.

The shape of this curve is essentially unaltered from month to month, but every point on the curve shifts with the seasons, being highest in the

spring and lowest in early January. As the result, the level of the entire curve rises and falls in an apparently regular pattern. This can be seen to advantage from figure 2, which shows for a 2-year period the shifts of one point on the curve; namely, the point for the 8th day of pseudopregnancy. The data for the two years do not fit perfectly, the 1938 spring peak occurring earlier than that of 1937. It may be interesting to note that in the spring of 1938 the changes in the animals at the zoological gardens and in the plant life were so unusually early as to call forth special comment from the daily newspapers.

Our data for estrous rabbits and for other stages of pseudopregnancy are less extensive, but they follow the same trend. The most striking change is in the glands at the 6th day of pseudopregnancy, which rise from a value of 7 in the autumn to 30 in the spring (no winter values available for this stage of pseudopregnancy). Estrous glands assayed 30 in the autumn months and 50 in the spring (spring glands obtained in March and May 1937).

In view of the relative constancy of the room temperature for the entire year, and the uniformity of the diet, we were obliged to consider light as the principal environmental factor. Yet, we were unable to increase the hormone content of autumn gland to the spring level by exposure of the donors to artificial sunlight (General Electric Sunlamp) or to ordinary electric light for 8 hours nightly starting at sundown. The light intensity in the cages under the Sunlamp was about 80 foot candles. In the cages lighted by batteries of 200 watt lamps the intensity averaged 45 foot candles. Several groups of animals killed at the 8th day of pseudopregnancy after 23 to 35 days of such treatment yielded pituitaries with a hormone content within the normal autumn range. The only suggestions of positive results were obtained with one group of animals exposed to the Sunlamp for 66 days and one group exposed to the electric lamps for 95 days. Although each of these groups consisted of only three animals their pituitaries, obtained in early January, contained twice as much gonadotropic activity as the control glands (40 U as opposed to 18 for the controls of that time of year).

DISCUSSION

The restitution of the hormonal content of the rabbit pituitary following post-coital discharge as presented in figure 1 differs somewhat from the results of Hill. Our data show very little progress in restitution before the 6th day of pseudopregnancy (except in the spring months). The graph presented by Hill shows much more progress by the 6th day. This author, however, used pituitaries from animals 24 hours after coitus for the first point of pseudo-pregnancy, but used pituitaries from animals injected with pregnancy urine for the other points of pseudopregnancy. Hill's data show that the discharge of the gland following PU injections is not so complete as that following coitus. If we should substitute in Hill's graph his assay of the glands 24 hours following PU injection for his assay of glands 24 hours post-coitum this graph would also show very little restitution of the gland content by the 6th day. A second point of difference between the two sets of data is found at the 10th day of pseudopregnancy. Hill found the gland content to

be higher at the 10th day of pseudopregnancy than at estrus. We found the 10-day glands to be about equal in content to the estrous glands. In view of our findings in regard to the seasonal variation, it would be interesting to know at what times of the year Hill's animals were killed.

This seasonal variation of pituitary content as found in our laboratory finds no plausible explanation other than some inherent rhythm in the species, perhaps modified by environmental factors. In respect to the reproductive system the rabbits were strictly comparable at all times of the year.[1] The diet was constant consisting only of pellets already described (6). The

TABLE 1. BODY WEIGHT AND PITUITARY GONADOTROPIC CONTENT OF DONORS OF GLANDS AT THE 8TH DAY OF PSEUDOPREGNANCY

Date	No.	Weight	Assay
9/21/37	3	3.07	<25
10/13/37*	4*	2.83*	18*
10/15/37	3	3.45	35
11/10/37	4	3.93	35
11/23/36	4	3.45	20
11/30/36	8	3.00	18
11/30/37	3	2.66	30
12/ 2/37	5	4.06	20
1/ 3/38	5	4.09	18
1/16/37	6	3.40	12
1/25/38	3	3.70	30
2/18/37	5	3.78	35
2/28/38	5	3.49	50
3/19/37	8	4.17	50
3/14/38	5	3.53	40
4/ 5/37	7	3.58	60
4/14/38	5	3.28	40
5/17/38	5	3.35	35

* By oversight the vials containing these glands were not placed in the refrigerator immediately after the autopsies but were left out in the warm room for 4 days.

(The dates refer to the time of coitus. Number refers to the number of animals in each group, and weight, to the average body weight of each group.)

data show no seasonal variation in the average pituitary weights of the groups of animals serving as donors (average of all group averages being 43; $\sigma = \pm 2$). The data did indicate that the donors in the spring of 1937 were somewhat heavier than the donors in the autumn of 1936. Although the assays did not reveal any obvious relation of body weight and pituitary content, the data of 1936–37 did not permit us to exclude body weight as a factor. In the autumn of 1937 we purposely chose small groups of donors whose body weights differed widely. Then, we made special efforts to obtain heavier animals for the following winter months, and lighter animals for the spring months. The extent to which this was done is shown in table 1. If there is any association between body weight and pituitary content, it is not indicated by this table. Certainly the correlation is not great enough to warrant serious consideration in this discussion.

There remain two variables: sensitivity of the assay animals, and some artificial selection of the donors occasioned by our methods of procurement.

[1] All were healthy post-partum females at the start of the experiment.

The sensitivity of the assay animals in regard to pregnant mare serum extract has been checked and found to be constant throughout the year (7). We cannot guarantee absolute uniformity of the donors. They were not of pure-bred stock, and were not even of the same race. A majority of the animals were chinchillas, but the rest were New Zealand Whites, Havana Browns, American Blues and New Zealand Browns. Nevertheless the records show no seasonal alteration in the proportions of these breeds among the donors used in these experiments. Unfortunately, we have no records of the age of the experimental animals. Nor have we any information about the details of the previous sexual history. All we have is the assurance from the rabbit breeder that he was unaware of any differences in the animals shipped in the spring and in the autumn. He had made no conscious selection on the basis of age, breed, or previous sexual history. Unless some unconscious basis of selection was operating in a very similar manner over the 2-year period, we are forced to attribute the observed variations in the pituitary to some genuine physiological rhythm. A similar rhythm has been reported in the pituitary of the ground squirrel (10).

Our inability to increase the pituitary content in the autumn months by artificial light does not argue against light as the most important environmental influence. Indeed the results from the two groups exposed to artificial light for 66 days or longer might be accepted as evidence for the importance of light. It might be recalled that even in the ferret it has not been possible to prevent the onset of anestrus by continued artificial lighting, or to prevent the occurrence of estrus by subjecting the animals to complete darkness (5, 9, 11).[2] Whatever the cause for the observed variations in hormonal content of the rabbit pituitary, these variations cannot be ignored in any future investigations in which such glands are used.

SUMMARY

Confirming the results of R. T. Hill, we have found that the gonadotropic hormone content of the rabbit pituitary is rapidly depleted in the 24 hours following mating, and that the restitution of the gland content follows a characteristic curve during pseudopregnancy. Repeated assays over a 2-year period of glands from estrous rabbits, and rabbits at several stages of pseudopregnancy have demonstrated a variation in the level of this entire curve, each point of which is highest in the early spring and lowest in early winter.

This work was supported by a grant from the Rockefeller Foundation.

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[2] Our own data on the effect of complete darkness on the rabbit pituitary are inconclusive.

DEPRESSION OF GASTRIC SECRETION BY THE ANTERIOR PITUITARY-LIKE FRACTION OF PREGNANCY URINE[1,2]

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THIS INVESTIGATION was prompted by the following observations: *a*), the acidity of the gastric contents tends to be reduced in women during pregnancy and rises during the puerperium (1, 2, 3, 4, 5, 6); *b*), the reduced acidity is not apparently due to enterogastric regurgitation (6); *c*), 'peptic' ulcer is rare in pregnant women and, when it has been present previously, relief from symptoms occurs during pregnancy (review of the literature, 7).

These observations raised the question of the cause of the reduced acidity of the gastric contents during pregnancy in women. The possibility that those hormones which are produced in excess during pregnancy might be concerned had to be considered. Estrone was suspected by Winkelstein (8), but has apparently been ruled out by the observations of Atkinson and Ivy (9) on dogs and man (using 10,000 R.U. daily) and of Schiff, Felson, Graff, and Meyer (10) on human subjects, although the daily dose (10,000 R.U.) used was only about one-fifth to one-sixth the maximal daily urinary output of estrogenic substances by the pregnant woman. Emmenin, or estriol glycuronide, has not been found to depress significantly the gastric secretion of patients (9). Manville and Munroe (11) failed to find that the hormone of the corpus luteum was concerned, although their evidence is very meager.

We chose the anterior pituitary-like fraction (APL) of human pregnancy urine for study because it is produced in great quantity during the earlier period of pregnancy in women when gastric secretion is reported to be most frequently depressed, whereas in the dog APL is not thought to be produced and gastric secretion seems not to be depressed during the early stages of pregnancy (12, 13, 14, 15). In mid-pregnancy, in the dog gastric secretion is reported to be slightly increased (14) or decreased (12, 13). Just before parturition it is reported to be increased in four dogs (13, 15, 16) and decreased in two (14, 17). Gastric secretion (volume output) was increased if the pups were nursed (13, 15, 17) or returned to normal when the pups were not nursed (16) or were nursed (12). Although the evidence on the effect of pregnancy on gastric secretion in the dog is meager and equivocal, achlorhydria

[1] This investigation was aided by a grant from Mr. E. L. Dawes.
[2] The antuitrin-S used was supplied through the courtesy of the Parke, Davis & Company; the follutein was furnished through the courtesy of the E. R. Squibb & Sons Company.

which is reported to occur not infrequently during pregnancy in women, has not been observed in the dog.

METHODS

Studies were made on 5 non-castrated healthy female dogs with Pavlov pouches, using a standard test-meal which consisted of water and cooked cereal and meat. The secretion was collected from the pouch for 30 to 60 minutes preceding the meal in order to be certain that the gastric secretory mechanism was in a basal state. Following the meal the secretion was collected for a 6-hour period, volume was noted, free and total acids were determined by titration with N/10 NaOH and expressed as the total number of mg. HCl for the entire 6-hour post cibal period.

An adequate series of control tests were made on each dog preceding each course of APL. During the control tests placebos of physiologically normal saline or of 20% aqueous glycerine solution were administered (the former preceding courses of antuitrin-S and the latter preceding courses of follutein).

Two preparations of anterior pituitary-like sex hormone were used: antuitrin-S (Parke, Davis & Company) and follutein (E. R. Squibb & Sons Company). Both are extracts of human pregnancy urine. The former is an aqueous solution and was administered subcutaneously; the latter is a glycerine solution (20% glycerine when prepared for injection) and was administered by intramuscular injection.

It was advised[3] that small (clinical) doses were ineffective on the gastric secretion of dogs, a finding which we had confirmed in a preliminary trial, so we used only large doses varying from 1000 to 5000 R.U.[4] daily for from 5 to 7 consecutive days. During this time daily tests of the gastric secretory response were made. Such tests were continued for appropriate periods after administration of APL was discontinued. The APL was injected one-half hour before feeding the test meal.

An 'inactive-control series of tests' was made; 5000 R.U. daily of APL inactivated by boiling the ampoule in a water bath for 90 minutes (18) were injected to determine whether or not the effect observed was specific. Similar treatment for 10 minutes did not inactivate the gastric-secretory-depressing substance.

Browne and Venning (19, 20) state that in pregnancy in the human, gonadotropic (APL) substance appears in the urine between the 9th and 15th days of the first missed period, that it reappears about the 25th day and increases gradually up to the 32nd day, at which time the rate of excretion is 200 to 500 R.U. per liter of urine. It then rises rapidly to a peak between the 52nd and 64th days, reaching an excretory rate as high as 133,000 to 400,000 R.U. per liter of urine. About the 67th day the rate drops sharply to about 40,000 R.U. per liter. On the basis of a woman weighing 150 pounds, our dogs received doses comparable to from 4000 to 50,000 R.U. of APL substance daily during the experimental period.

RESULTS

Before performing the experiments with the APL fraction of human pregnancy urine, it was decided to test desiccated whole human placenta and emmenin on dogs with Pavlov pouches.

Desiccated whole placenta. A number of human placentas were collected and desiccated at low temperature in vacuo.[5] 100 gm. and 50 gm. (1.0 gm. equivalent to 6.86 gm. of fresh tissue) were fed daily to 2 dogs, with Pavlov

[3] Dr. D. J. Sandweiss (by personal communication).

[4] One rat unit is defined as the minimum amount which, given in 6 injections on 3 consecutive days, produces mature follicles, hemorrhagic follicles and corpora lutea in the ovary within 100 hours after the first injection in 30-day-old female rats.

[5] We desire to thank Dr. David Klein of the Wilson Laboratories, Chicago, for this courtesy.

pouches, respectively for 31 days. A depression of gastric secretion was not observed; one of the dogs showed an increase in secretion that could be attributed to the increase in protein intake.

Emmenin. Twelve cc. (60 day-oral estrogenic units) of emmenin[6] (23) daily were given orally to 2 pouch dogs for 18 days. Gastric secretion was not affected.

Anterior pituitary-like fraction. The data are too bulky to present in detail. The results of a series of tests on dog 3 are given in table 1, from which

TABLE 1. PROTOCOL ON DOG 3, SERIES II

		Volume in cc.	Free acid in mg. HCl	Total acid in mg. HCl
Controls				
7/15/38	Placebo and meal	36.8	64.17	74.02
7/16	Placebo and meal	38.7	57.60	70.73
7/17	Placebo and meal	37.9	39.74	53.24
7/19	Placebo and meal	46.9	78.75	97.35
	Mean with probable error	40.1±1.6	60.06±5.4	73.83±6.1
7/20/38	5000 R.U. follutein; meal	14.3	—	5.81
7/21	5000 R.U. follutein; meal	13.6	0.72	4.18
7/22	5000 R.U. follutein; meal	21.9	17.67	25.87
7/23	5000 R.U. follutein; meal	17.7	6.19	12.03
7/24	5000 R.U. follutein; meal	25.7	16.77	24.96
	Mean with probable error	18.6±1.6	8.27±2.6	14.57±2.8
Follow-up				
7/26	Meal	41.7	68.18	86.41
7/27	Meal	36.1	41.93	61.63
7/28	Meal	42.1	55.42	70.37
	Mean with probable error	39.9±1.3	55.17±5.1	72.80±4.9
8/11–8/15	Vaginal bleeding			

are omitted the results of a number of previous controls and of a more extensive follow-up period. The rest of the data are summarized in table 2 and illustrate the essential observations.

In the case of dog 1 the figures given for the 'follow-up' were those obtained for 28 days after the injections were stopped. About 45 days later the values had risen to the control values for series II. The second series of injections were started about two months after the first, the animal having remained constant at this 'new' higher level. Following this second series of injections the secretion of the dog rapidly recovered. The tests in the inactivated control series were made 2½ months after series II.

In the case of dog 2, the first and second series of injections were separated by only 8 days. The 'follow-up period was only 13 days in length because of the accidental prolapse of the pouch, which, even though corrected, renders the pouch worthless until a new series of controls are obtained.

In the case of dog 3, the 'follow-up' period after the first series of injections constituted the first 7 days. It is to be noted that following the injec-

[6] Obtained through the courtesy of Ayerst, McKenna and Harrison Company.

TABLE 2. SUMMARIZED RESULTS OF EXPERIMENTS USING APL FRACTION OF PREGNANCY URINE TO DEPRESS GASTRIC ACIDITY

		Volume in cc.	% of Contr.	Free acid in mg. HCl	% of Contr.	Total acid in mg. HCl	% of Contr.	
Dog 1	Wt.=33 lbs.							
Ser. I	Control	31.5±2.0	100.0	106.3± 5.8	100.0	128.9± 5.1	100.0	5 days
	Antuitrin-S							
	1000–2000 R.U.[1]	17.4±1.8	55.3	50.9± 8.1	47.9	59.3± 8.7	46.0	5 days
	Follow-up[3] 1)	22.2±2.3	70.4	74.2± 8.2	69.8	83.9± 8.5	65.1	28 days
	2)	57.3±3.5	181.9	209.4±21.6	197.0	228.5±22.7	177.3	4 days–(45 days later)
								2 mo. later
Ser. II	Control	57.3±3.5	100.0	209.4±21.6	100.0	228.5±22.7	100.0	4 days
	Follutein							
	5000 R.U.[1]	31.3±1.8	54.6	100.5±12.6	47.9	110.5±19.1	48.3	5 days
	Follow-up	59.6±0.9	104.0	237.8± 4.2	113.5	258.7± 3.8	113.2	3 days
Ser. III	Control	49.6±1.0	100.0	169.2± 2.6	100.0	191.0± 1.7	100.0	5 days
	Inactivated Follutein, eq.[2]							
	of 5000 R.U.	47.3±0.9	95.3	167.9± 2.4	99.2	186.8± 2.8	97.7	5 days
Dog 2	Wt.=35 lbs.							
Ser. I	Control	22.1±0.8	100.0	48.1± 2.1	100.0	56.1± 3.0	100.0	5 days
	Antuitrin-S							
	1000 R.U.[1]	12.9±1.4	58.3	15.9± 4.9	33.1	21.7± 4.9	38.7	5 days
	Follow-up	22.5±3.2	101.8	40.4± 8.7	84.0	52.6±11.2	93.7	5 days
Ser. II	Control	22.5±3.2	100.0	40.4± 8.7	100.0	52.6±11.2	100.0	5 days
	Antuitrin-S							
	1000 R.U.[2]	13.9±0.8	61.7	16.3± 3.3	40.4	20.6± 3.3	39.1	7 days
	Follow-up	13.1±1.2	58.2	17.8± 4.4	44.1	24.4± 5.4	38.7	13 days
Dog 3	Wt.=18½ lbs.							
Ser. I	Control	18.8±2.1	100.0	35.4± 5.0	100.0	44.2± 5.7	100.0	15 days
	Follutein							
	5000 R.U.[1]	14.4±3.1	76.5	14.4± 6.6	40.7	20.8± 8.9	47.1	5 days
	Follow-up	31.3±2.8	166.4	46.3± 6.6	130.9	68.9± 8.9	155.7	7 days
								2 mo. later
Ser. II	Control	40.1±1.6	100.0	60.0± 5.4	100.0	73.8± 6.1	100.0	4 days
	Follutein							
	5000 R.U.[1]	18.6±1.6	46.3	8.2± 2.6	13.7	14.5± 2.8	19.7	5 days
	Follow-up[3]	39.9±1.3	99.5	55.1± 5.1	91.8	72.8± 4.9	98.6	3 days
Dog 4	Wt.=15 lbs.							
	Control	45.1±3.9	100.0	108.1±18.5	100.0	139.0±19.5	100.0	8 days
	Follutein							
	5000 R.U.[1]	30.7±3.2	68.0	91.2±11.5	84.3	106.2±11.8	76.4	5 days
	Follow-up[3]	35.6±2.3	78.9	106.0±12.5	98.0	120.9±13.0	86.9	18 days
Dog 5	Wt.=20 lbs.							
	Control	35.7±4.0	100.0	47.9± 5.7	100.0	76.7± 6.6	100.0	5 days
	Follutein							
	5000 R.U.[1]	10.3±1.7	28.8	2.2± 1.1	4.7	7.9± 2.1	10.3	5 days
	Follow-up[3] 1)	23.5±1.3	65.8	28.4± 4.6	59.2	37.7± 4.7	49.1	17 days
	2)[4]	29.5±2.3	82.6	56.3± 6.2	117.5	66.6± 6.4	86.7	last 9 days (9–17)

The data are means with the probable error.

[1] In each instance this amount injected daily for 5 successive days.

[2] This amount injected daily for 7 successive days.

[3] Bleeding from vagina occurred during follow-up:

Time of year

Dog 1, series I in May
Dog 3, series II in August
Dog 4, in July
Dog 5, in July

[4] For Dog 5: Follow-up values returned to control level before end of follow-up; the values are given means for follow-up period.

tions the secretory level was higher than the controls. This higher level continued for 2 months at which time the second series of injections was given. Following the second series of injections the recovery was again prompt.

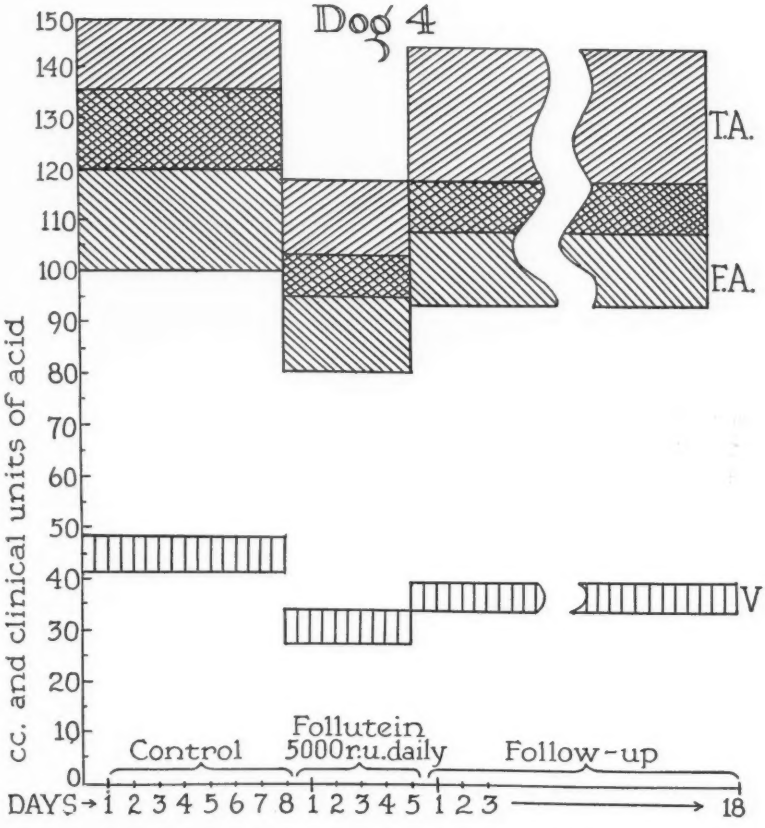

Fig. 1. 'T. A.,' TOTAL ACID IN MG.; 'F. A.,' FREE ACID IN MG.; 'V' VOLUME IN CC.

The 'follow-up' period on dog 4 was 18 days in length, at the end of which time the secretory level had not returned to the control level. In dog 5 the 'follow-up' period was 17 days in length. During the last 9 days of the period, the secretory level had returned practically to the control level.

Dog 3, under treatment manifested a complete achlorhydria for 3 days, as did dog 5 also. The minimum free acid value under treatment for dog 2 was 0.4 mg. HCl and for dog 1 it was 16.9 mg.; for dog 4, which showed but little depression, the minimum was 52.1 mg.

Upon statistical analysis the data lose some of the significance they appear to possess in the rough. Statistically significant depressions of secretion under treatment are found in 5 of the 8 experiments. In 2 of the other 3 ex-

periments the depression only very narrowly misses being statistically significant. Only in one experiment (dog 4) is the degree of the depression insignificant. By the criteria of most investigators the results on dog 4 would be considered significant (fig. 1). Thus, in 7 out of the 8 experiments in which an active preparation of the APL fraction was used a physiologically significant depression of gastric secretion was obtained.

It is to be noted that the gastric secretory depressing agent in the APL fraction of human pregnancy urine was inactivated by boiling the solution in the ampoule in a water bath for 90 minutes. Similar treatment for 10 minutes did not inactivate it.

The recovery from the depressing action of the hormone was relatively prompt in 4 of these 7 experiments and relatively slow in the other three. In two experiments the recovery was slow (dog 1, series I; and dog 2, series II). The rate of recovery was not correlated with the presence or absence of bleeding from the vagina which was associated with other manifestations indistinguishable from estrus or 'heat' in the dog. The bleeding (see table 2) appeared from 5 to 18 days after the first day of administration of APL and persisted for from 3 to 5 days. It was suspected that the slow return of the gastric secretion to the control level in one of the experiments (dog 1, series I) was due to a pseudopregnancy. Exploration was not done because it was not desirable to disturb the animal. It should be pointed out that in the experiment just referred to (dog 1, series I) a delayed secretion was observed in that the secretion continued up to the 8th hour post-cibum period rather than to the usual 6th hour period.

DISCUSSION

The results show unequivocally that a depression of gastric secretion occurs in the female dog when 1000 to 5000 R. U. of the APL fraction of human pregnancy urine are injected daily one-half hour before feeding the test meal. The results throw no light on the mechanisms involved. It cannot be said that the active principle acted directly on the parietal cells of the stomach, or produced the effect on the parietal cells by way of the ovaries, uterus, adrenals, hypophysis, or some other organ in the body. It would be desirable to extend this investigation to include male dogs and spayed and hypophysectomized females, provided the active principle in this fraction of urine is actually APL. De Lisi (21) reports some observations which would implicate the thyroid and ovaries as intermediates when estrogen and the gonadotropic principle of the hypophysis are given to dogs with Pavlov pouches; but his methods and data appear equivocal to us. The immediate response to the APL substance and the prompt return in 4 of the 7 experiments would argue in favor of a direct action of the substance on the parietal cells. The slow return of the stomach to the control level would argue in favor of an indirect action. Our results suggest that the effective substance or substances in the APL fraction acted on the parietal cells both directly and indirectly, chiefly directly.

Some data obtained on dog 1, series II, suggested that the secretion was being delayed or only temporarily inhibited, thus being analogous to the temporary depression that occurs after a single injection of atropine or entero-

gastrone (22). Under control conditions the gastric secretory response to a meal is practically complete in 6 hours; but in some instances after the injection of APL the animal secreted more during the sixth to eighth hours than during any of the others.

The possibility that the gastric secretory inhibiting substance in the APL fraction is enterogastrone cannot be ruled out on the basis of existing knowledge regarding the chemical properties of the two substances (22, 23). The solution to this problem should prove to be of much physiological and clinical interest (7, 24). The heat lability of the APL sex hormone suggests that the gastric secretory inhibiting agent in the APL fraction of urine is the gastric secretory inhibiting fraction of enterogastrone.

SUMMARY

A study of the effect of the parenteral administration of the APL fraction of human pregnancy urine on the gastric secretory response to a test meal was made in 5 female dogs with Pavlov pouches. After suitable control tests, from 1000 to 2000 R.U. of antuitrin-S and 5000 U. of follutein were given parenterally one-half hour before the test meal. In 4 of the 5 dogs, in 7 out of 8 tests, significant to marked decreases in volume and acid output occurred each day during a 5-day period of treatment. In 4 of the 7 instances the secretory response returned to normal one day after cessation of the treatment; in the other 3 the return was slower. 'Estrus' occurred in 4 of 8 instances from 5 to 18 days after the administration of the first dose of APL. Heating the ampoule containing the APL fraction for 90 minutes at 100° C. inactivated the substance which depressed gastric secretion; heating similarly for 10 minutes did not inactivate it.

It is concluded that the APL fraction of human pregnancy urine contains a substance which depresses the gastric secretory response of a dog to a meal. The nature of the substance and its mechanism of action is problematic.

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THE RELATION OF THE PITUITARY TO BLOOD LIPIDS[1]

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BURN AND LING (1, 2) and Anselmino and Hoffman (3) demonstrated that extracts of the anterior pituitary produced a ketosis in rats or rabbits which were fasting or receiving a high fat diet. That this effect was not mediated through the thyroid was indicated by Funk (4) who observed that thyroidectomized rats responded to AP extracts similar to normal animals and further Butts, Cutler and Deuel (5) reported little ketonuria following injections of the thyrotropic hormone. On the other hand, MacKay and Barnes (6) and Fry (7) report a lack of ketosis in adrenalectomized rats injected with a ketogenic AP extract. Mirsky (8) concluded that the adrenal gland by itself is not essential for the ketogenic activity of extracts of the AP, but rather an increase in the renal threshold for ketone bodies is responsible for the decreased ketonuria.

Best and Campbell (9) reported intense fatty infiltration of the liver of fasted white rats following the daily administration of an AP extract. These investigators (10) later noted a similar effect in mice and guinea pigs.

An increase in blood lipids was reported by Evans (11) and Munoz (12, 13). Strauber (14) noted a decrease in blood lipid following injection of an AP extract.

In this laboratory our interest is centered around the relation of the pituitary hormones to lactation. Considering the large amount of fat in milk, there can be but little question that the metabolism of fat plays an important rôle in milk fat secretion. The experimental evidence now at hand indicates that the precursors of milk fat consist principally of neutral fat and fatty acids. If the fat metabolism hormone influences the amount of these lipids in the blood it seemed desirable to learn its effect upon experimental animals, to work out an assay method for its determination and to test out methods of separation and chemical concentration.

The object of the present paper is to report the results of our study of the influence of several pituitary extracts upon the fat content of rabbit blood.

Experimental procedure. New Zealand White female rabbits weighing from 2.5 to 3.5 kg. were used. Each animal was kept in a separate cage to prevent pseudo-pregnancy. Blood was obtained from an ear vein at the be-

[1] This study has been aided in part by grants from the Committee on Research in Endocrinology of the National Research Council. Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series, No. 592.

ginning of the experiment (as a normal control) and usually at 2-hour intervals after the AP extract had been injected intraperitoneally. Each successive bleeding was made slightly distal to the last cut. During the experimental period, food was withheld from the animals. In the fasting experiment food was withheld for 24 hours before its start.

The oxalated blood was centrifuged and the plasma fat was determined by the method described by Allen (15, 16). Included in this determination are the neutral fats, sterols, sterol esters and lipochromes. Phospholipids are not included. Duplicate and triplicate determinations checked within 5 mg. per cent.

TABLE 1. PLASMA FAT CONTENT OF UNINJECTED RABBITS
(Normal Controls)

Exp. No.	Body wt. kg.	Blood Samples Taken							
		Initial bleeding mg. %	2 hr. mg. %	4 hr. mg. %	6 hr. mg. %	8 hr. mg. %	10 hr. mg. %	12 hr. mg. %	24 hr. mg. %
1	2.8	420	410	420	420	425			425
2	3.6	195	190	185	190	190			185
3	2.5	250		240	230			225	250
4	2.8	190		190		175	180		185
5	2.6	190	190	195	190	185	205		185
6	2.8	225	260	260	240	250	230		
7	2.7	170	187	180	180	200	190		
8	2.8	300	305	290	310	285	302	310	
9	3.0	320	310	300	310	310	290	280	
10	3.0	505	490	510	495	500	500		520
Average		277	293	277	285	280	271	270	290

The AP extracts used in the experiments here reported consisted first in the initial extract prepared by Bergman and Turner (17). This extract is known to be rich in lactogenic, carbohydrate metabolism, gonadotropic and thyrotropic hormones. The initial extract has been further separated by Bergman and Turner (18) into lactogenic and carbohydrate-metabolism fractions. The former has been found to be quite free of the other above hormones whereas the latter contains the gonadotropic and thyrotropic hormones as well. For purposes of injection, the dried preparation was dissolved in a slightly alkaline solution (with NaOH) then precipitated by bringing the solution to the isoelectric point with HCl.

Normal blood fat of the rabbit. The normal blood fat in the rabbit has been reported by Boyd (19) to vary from 81 to 482 mg. per cent. Sex, season, body weight, and time of day (not diurnal variation) were found to have little influence upon the blood fat content.

To compare with groups of experimental animals, a group of 10 rabbits weighing from 2.5 to 3.0 kg. was selected and the plasma fat determined at intervals of two hours for 12 hours and again at 24 hours (table 1). The average fat content in 62 samples was 281 mg. per cent with a range from 190 to 505 mg. per cent. In all cases the first samples were taken between 8 and 10 A.M. Neither body weight nor interval of bleeding seemed to affect the blood fat content (fig. 1).

EXPERIMENTAL

In a group of 14 rabbits comparable to the normal controls, a blood sample was taken, then each animal was injected with 300 mg. of the initial extract. Samples of blood were then withdrawn at intervals up to 24 hours (table 2). Plasma fat determinations of these samples showed that, on the average, a slight decline in fat had occurred within 2 hours. After 4 hours there was an average decrease of 28% and after 6 hours a decline of 36% of the initial average value. At this time no individual showed a decline of less than 27%. The blood fat continued low for 2 hours (the 8-hour period) after which there was a gradual increase reaching approximately normal 24 hours after the injection.

TABLE 2. THE EFFECT OF AP EXTRACTS ON THE PLASMA FAT OF RABBITS
(Injection of 300 mg. per animal)

Exp. No.	Body wt. kg.	Normal mg. %	Interval after Injection											
			2 hr.	4 hr.		6 hr.		8 hr.		10 hr.		12 hr.		24 hr.
			mg. %	mg. %	Decrease %	mg. %	Decrease %	mg. %	Decrease %	mg. %	Decrease %	mg. %	Decrease %	mg. %
1	2.5	270						195	28.0					
2	2.5	240	220	214	10.0	160	33.3	150	37.5	160	33.3			203
3	2.8	340	310	250	26.4	235	30.9	250	26.4	240	29.4			300
4	2.5	410		300	26.8			305	23.3	390				470
5	2.6	290	275	200	31.0	185	36.2	190	34.5	195	32.8			310
6	2.5	450		320	28.9	250	44.4	240	46.7	260	42.2			460
7	2.7	250		95	62.0	80	68.0	160	36.0					275
8	2.9	360		280	22.2	250	30.5	240	33.3	260	27.8			350
9	3.0	555		330	40.5	400	27.9	400	27.9	420	24.3	430	22.6	555
10	2.8	830		700	15.7	600	27.7	580	30.0	620	25.3			780
11	3.1	480		450		320	33.3	280	41.7	300	37.5			375
12	3.0	360		300	16.7	250	30.6	225	37.5	230	36.1	240	33.1	355
13	3.2	380						250	34.2					
14	2.7	600						410	31.6					
Average		417.5		313	28.0	272.0	36.3	277.0	33.5	307.5	32.1	335	27.9	403

In a second group of 6 rabbits, food was withheld for 24 hours previous to the time of the first bleeding. The preinjection blood fat was found to range from 225 to 540 mg. per cent with an average of 354 mg. per cent (table 3). These observations indicate that withholding food for 24 hours does not affect blood fat to any material extent. After taking a blood sample, 300 mg. of initial extract was injected into each animal as in the previous experiment. The average percentage decline in blood fat was quite similar to that observed in the previous group. The greatest decline was observed during the period from 6 to 10 hours after the injection, with a subsequent return to normal within 24 hours.

These observations indicate that the initial extract of the AP contained a factor which definitely depressed the blood plasma fat of both normal and 24-hour fasted rabbits during a period of 6 to 10 hours after intraperitoneal administration. For some as yet unknown reason the preinjection blood fat

content of some of the experimental rabbits were quite high thus raising the average of these groups considerably above the uninjected animals. For comparison, the average percentage change in blood fat from the preinjection

TABLE 3. THE ACTION OF AP EXTRACTS ON PLASMA FAT, FEED BEING WITHHELD
(Injection of 300 mg. per animal)

Exp. No.	Body wt. kg.	Normal mg. %	Interval after Injection									
			2 hr.	4 hr.		6 hr.		8 hr.		10 hr.		24 hr.
			mg. %	mg. %	Decrease %	mg. %	Decrease %	mg. %	Decrease %	mg. %	Decrease %	mg. %
1	2.5	325	285	230	29.2	220	32.3			220	32.3	300
2	2.9	225	220	180	20.0	160	28.9	140	37.8	80	64.5	200
3	2.6	325	295	260	20.0	180	44.6	210	35.4	220	32.3	310
4	2.8	280	280	210	25.0	175	37.5	190	32.1	200	28.6	290
5	2.3	420	410	350	16.7	300	28.6	325	22.6	340	19.0	500
6	2.5	540	450	420	22.2							
Average		354	323	273	22.2	207	34.4	216	32.0	212	35.3	320

level seems to be most satisfactory (fig. 1). It should be noted, however, that the percentage decline of plasma fat was of approximately the same magnitude whether the initial level was low or high.

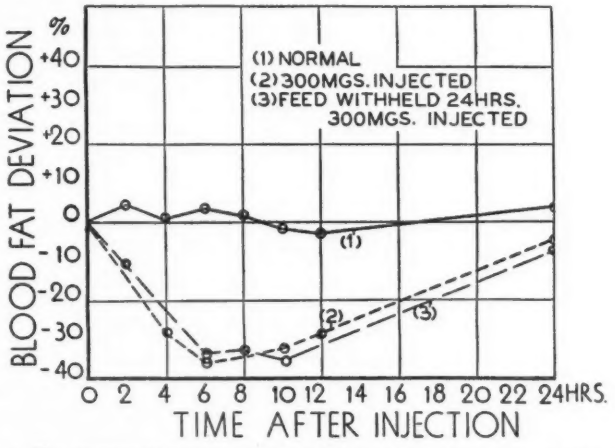

Fig. 1. THE PERCENTAGE DEVIATION OF THE BLOOD FAT OF NORMAL RABBITS IN RELATION TO THAT OF THOSE INJECTED WITH APE

From the above data it appears that the initial extract of the AP contains a factor which influences fat metabolism as indicated by its effect upon the plasma lipids. In order to determine whether it is identical with some of the other recognized pituitary hormones, a study was made of the effect of certain fractions of the above extract prepared by Bergman and Turner (18). The procedure has been described for separating the lactogenic hormone

from the blood-sugar-elevating principle. Work in this laboratory indicates that the lactogenic fraction is quite free of the carbohydrate metabolism, thyrotropic and gonadotropic factors whereas the carbohydrate metabolism fraction contains the thyrotropic and gonadotropic factors as well.

TABLE 4. THE EFFECT OF FRACTIONS OF AP EXTRACTS ON PLASMA FAT OF THE RABBIT

Hormone (fraction administered)	Amount	No. of animals	Ave. body wt. of animals	Blood fat (mg. %) ave.	
				Normal	8 hours
	mg.		*kg.*		
Carbohydrate-metabolism	300	7	2.93	257	260
Carbohydrate-metabolism	600	2	2.85	350	340
Lactogenic	300	8	2.96	219	214
Lactogenic	600	2	3.00	220	235

In order to determine if the fat metabolism hormone was associated with either of these two fractions, groups of rabbits were given 300 mg. of these fractions; the same amount as was found previously to cause a marked lowering of the plasma fat. Since these fractions represent a considerable concentration of the hormone in the initial extract, this amount should have

TABLE 5. THE EFFECT OF ADRENAL CORTEX EXTRACT (WILSON) ON THE PLASMA FAT OF THE RABBIT

Exp. No.	Amount given	Normal mg. %	4 hr. mg. %	6 hr. mg. %	8 hr. mg. %
	cc.				
1	0.2	310	300		305
2	0.2	285	290		280
3	0.2	170	170		175
4	0.2	310	290		315
5	0.2	390	345	350	370
6	0.2	195	180	210	190
7	0.2	320	210	295	240
8	0.2	110	160	205	197
9	1.0	60	40		90
10	1.0	90	80		90
11	1.0	130	110		75
12	1.0	100	80		85
13	2.0	95	120		80
14	2.0	90	105		90

produced a very marked effect if the fat metabolism factor was identical or associated with the above hormones. Instead neither fraction at the 300 mg. level showed a decrease in plasma fat 8 hours after the administration of the hormone (table 4). Two groups of 2 rabbits each were then injected with 600 mg. of these 2 fractions respectively. As no response was noted, it seems reasonable to conclude that the fat metabolism hormone is an entity distinguished from the above mentioned AP hormones. Further, this factor appears to be removed in the concentration of these 2 extracts. Other fractions of the initial extract are now being investigated.

Since it has been indicated (6, 7) that adrenalectomy influences ketosis, it seemed of interest to determine the effect of the adrenal cortical hormone upon the plasma fat of the rabbit. Increasing amounts of adrenal cortex extract (Wilson) were without effect upon 14 rabbits injected (table 5).

It is well known that thyroxine increases the metabolism of the body. Increased metabolism in turn might be expected to decrease the lipid content of the blood. In fact Graham et al. (20) showed in dairy cattle a marked decrease of the fatty acids in arterial blood during continued thyroxine administration. The injection of 5 mg. of thyroxine each into 10 rabbits showed

TABLE 6. THE EFFECT OF THYROXINE ON PLASMA FAT OF THE RABBIT

Exp. No.	Amount given	Normal mg. %	6 hr. mg. %	8 hr. mg. %	10 hr. mg. %	22 hr. mg. %	30 hr. mg. %
	mg.						
1	5	155	220	190	145		
2	5	195	100	70	70		
3	5	330	350	380	340		
4	5	130	125	125	125		
5	5	270	155	145	145		
6	5	145	125	125	195		
7	5	280	250			110	100
8	5	230	230			230	160
9	5	250	180			185	120
10	5	150	150			105	80

little effect during a period of 6 to 10 hours after administration, however, a definite decline was observed in blood samples taken from 22 to 30 hours later. It is clear that the extract of the AP which depresses plasma fat is not acting through the thyroid gland (table 6).

DISCUSSION

The first evidence of an anterior pituitary factor playing a rôle in the complicated series of transformations which may be included under the term "fat metabolism" involved ketonemia. Subsequently it was shown that a ketonuria resulted. As it is generally recognized that ketone bodies are produced by metabolism of fats in the liver, it was not unexpected to find a fatty infiltration of the liver to follow AP extract administration. If the fat was transported to the liver by the blood, evidence of this should be observed by alteration of the lipid content of the blood. The data presented in this paper clearly indicate that the blood fat is altered by the AP extract used but it may seem surprising that the immediate effect is to produce a fall in the fat content rather than a rise as was observed in the case of the carbohydrate metabolism factor. It might be possible that the hormone stimulates the uptake of fat by the liver at a more rapid rate than the inflow to the blood from digestion or fat depots. On the other hand, it might be thought that the decrease of blood fat was inversely related to the increase in blood sugar due to carbohydrate metabolism hormone. However, the observation that

the carbohydrate metabolism fraction was ineffective in depressing the plasma fat is evidence for the separate identity of carbohydrate and fat metabolism hormones.

SUMMARY

The plasma fat of a group of 10 New Zealand White rabbits was determined at 2-hour intervals for 12 hours and again after 24 hours. The average fat content remained rather uniform during the period observed.

In a second group of similar animals, blood samples were taken, then 300 mg. of Bergman and Turner's initial extract of the AP were injected intraperitoneally into each. Samples of blood taken at intervals showed a gradual decline reaching an average of about 36% (below the initial sample) within 6 to 8 hours, followed by a return to approximately normal after 24 hours.

Similar results were observed in another group of rabbits from which food had been withheld for 24 hours previous to the time of injection. The curves of fat depression were parallel in the two experimental groups.

Fractions of the initial extract rich in lactogenic hormone and in the carbohydrate metabolism, thyrotropic and gonadotropic hormones respectively were next injected at the same level. In neither case was a depression of the plasma lipids observed indicating that the fat metabolism hormone is neither the same nor associated with these two groups of AP hormones.

To determine whether other hormones would influence blood fat, adrenal cortex extract (Wilson) and thyroxine were administered to groups of rabbits. Even at the maximum level, neither preparation showed an effect upon the blood fat within 6 to 10 hours. Thyroxine did begin to depress the fat after 24 hours.

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THE EFFECT OF THE ESTROGENIC HORMONE ON EXPERIMENTAL TUBERCULOSIS

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FOR MANY years the course of certain infections has been observed to vary periodically with the menses. It is particularly well known that some skin affections suffer an exacerbation before the menstrual periods. Herpes labialis is especially common at this time, in some cases occurring with great regularity. Dermatitis of the scalp may vary in the same manner. Acne vulgaris is prone to occur at the menarche, and frequently is worse preceding the menstrual periods. There are a number of papers relating to 'acute menstrual articular rheumatism' and to premenstrual exacerbations of erysipelas. Numerous other examples of menstrual exacerbations of acute infectious diseases have been reported. In some the relation is well known, in others less obvious. Much of the literature has been summarized by Novak (1) in his monograph on menstruation.

Perhaps the most interesting association is that of tuberculosis. Many observers for many years have noted the occurrence of premenstrual fever during the course of pulmonary tuberculosis. Statistics and curves illustrating this premenstrual elevation of temperature may be found in the papers by Brecke (2), Wiese (3), and Beekman (4). Furthermore, it has been widely recognized that in women in whom amenorrhea does not occur as a result of the disease, the condition of the patient may become periodically aggravated at each menstrual period. The cough may be increased in intensity; râles may become more widely distributed; and the tendency to hemoptysis may be increased. It is generally agreed that it is in the premenstrual period that these adverse signs most often make their appearance.

In addition to these cyclic effects observed in many women with active tuberculosis, it is of interest that whereas the mortality from tuberculosis is about equal in males and females up to the age of puberty, with the onset of menstruation the mortality rate in the female definitely out-strips that of the male, and tends to remain higher until the end of the active reproductive period.

These circumstances indicate that something peculiar to women during the reproductive life increases their susceptibility to tuberculosis and the exacerbation of infections of various other types in the premenstrual period suggests that resistance to these infections also may be affected by the cyclic play of the sex hormones. It is desirable, therefore, to acquire information

regarding the effect of these hormones upon resistance, and particularly in relation to tuberculosis.

Since one of the most prominent cyclic hormonal variations in the female is a pronounced rise in the level of estrogenic substance in the blood, and since this rise reaches its height in the period of the cycle in which the adverse effects of menstruation upon tuberculosis and other infections are most prominent (i.e. the premenstrual period) it was thought advisable to study first the effect of the estrogenic substance upon native and acquired resistance to tuberculosis. Studies on the effect of other hormones will be reported later.

Studies on the effect of the sex hormones on resistance to tuberculosis are meagre, and we have been able to find none in which the problem relating to the effect of estrogenic substance has been explored from the standpoint of acquired immunity. In 1931, Addessi (5) reported experiments in which he subjected rabbits infected with bovine tubercle bacilli to injections of urine of pregnant women. The rabbits receiving the urine died earlier than the controls, but there was no convincing difference in the extent of lesions. Aside from the fact that pregnant urine contains hormones other than estrin, Addessi himself did not regard his experiments as complete enough to warrant a conclusion regarding the effect of the estrogenic substance on tuberculosis.

In 1933, Vercesi and Merenda (6) treated tuberculous animals with injections of estrin every third day. Some of the animals received the hormone from the beginning of the infection, and others after 'symptoms of tuberculosis' had appeared. No definite difference was found between the animals that received the hormone and their controls.

In 1935, Repetti (7) treated tuberculous animals from the beginning of the infection with estrin and noted no difference in the development of tuberculosis in them as compared with untreated controls. The bacillus used, however, was of very low virulence, the treatment with estrin was stopped as soon as signs of established infection (local lesion, loss of vivacity, or weight) appeared, and no mention is made as to whether or not sufficient hormone was given to produce any visible genital effect.

In 1936, Bourgeois and Boguet (8) gave 1000 units of folliculin bi-weekly to 5 female guinea pigs, to 5 castrated females, and to 5 castrated males. Bovine bacilli were used to produce tuberculosis in all of them. The folliculin seemed to show no effect on the tuberculosis.

In none of the above experiments was any study made of the effect of the hormone upon acquired resistance. In the human adult with tuberculosis the factor of acquired resistance and its fluctuations under various adverse conditions is highly important. In the present study, therefore, it was our purpose to determine whether the estrogenic substance exerts any effect upon either native or acquired resistance.

The estrogenic substance employed in this study was the commercial preparation progynon-B in sesame oil, obtained through the courtesy of the Schering Corporation. The hormone was diluted in sesame oil, so that 0.1 cc. contained 20 R.U. In priming the animals in the first experiment a dose of 0.25 cc. or 50 R.U., was injected subcutaneously daily. After 10 days the animals developed a marked brawny edema with stiffening of the extrem-

ities, followed by death. A study of this condition will be reported in another paper. Consequently the dose was reduced to 0.1 cc. or 20 R.U., 3 times weekly throughout the experiment. Three doses were given before infection in order to prime the estrogenic group. The gross effect in all animals receiving the hormone was seen in the swelling of the vulva, which was maintained throughout the experiment. Vaginal smears showed full estrus. At autopsy all animals that received the hormone showed markedly enlarged uteri, cornification of the vaginal epithelium, hyperplasia of the uterine mucosa and hypersecretion of the cervical glands.

In the first series, designed to test the effect of the hormone upon native resistance, 30 virgin female guinea pigs were injected subcutaneously with 0.1 cc. of a suspension of human type bacilli, which showed an average of 3 bacilli per oil immersion field. Ten of the animals had been brought into estrus before the injection of the bacilli, as described above, and these continued to receive the hormone in the stated dosage throughout the experiment. Ten received 0.1 cc. of pure sesame oil, and 10 received 0.1 cc. of saline alone. All animals were injected on the same days 3 times a week.

Tuberculin tests with 1 and 5 mg. of O.T. made 33, 55, and 106 days after infection showed well marked reactions with no significant difference between the three groups.

Whenever an animal receiving the hormone died, an animal from the oil and the saline control groups was killed for comparison. Six of the hormone group lived 8 weeks or longer. Careful gross and microscopic examination was made of the local lesions, lymph nodes, and organs. There was no significant difference in the amount or character of the tuberculous lesions in the hormone-treated group as compared with the control groups.

The second part of this study dealt with the question of whether or not acquired immunity to tuberculosis might be affected by hyperestrinism. Forty-two female virgin guinea pigs were each given 0.5 cc. of a suspension of attenuated human type tubercle bacilli (Saranac R 1) in the right groin. Twenty-six days later all of the animals gave strong reactions to 5 mg. of O.T. injected intradermally. Twenty-seven days later after the immunizing dose of tubercle bacilli was given, the animals were divided into 3 groups, the first of which (18 animals) received 0.1 cc. of progynon-B containing 20 R.U., the second (12 animals) 0.1 cc. of sesame oil and the third group (12 animals) 0.1 cc. of normal saline. The solutions were injected subcutaneously 3 times a week. Two weeks after the injections were begun the genitalia of the group receiving progynon-B showed a pronounced estrogenic effect. Tuberculin tests repeated at this time showed no significant difference from the previous test in any group.

Forty-nine days after the immunizing bacilli were given, 0.2 cc. of a suspension containing 1/50 mg. of virulent human type tubercle bacilli was injected subcutaneously into the groin of each of the 42 animals, and in addition into 12 other female virgin guinea pigs which had not been immunized. The first 18 immunized animals continued to receive 20 R.U. of progynon-B in sesame oil subcutaneously, the second 12 received 0.1 cc.

sesame oil, and the third group of 12 immunized animals, and also the 12 non-immunized controls received 0.1 cc. normal salt solution. The injections were given every third day. Thirty days after receiving the virulent bacilli, although the group receiving progynon-B had shown continually a pronounced vulval effect, the hormone was increased to 40 R.U. in 0.1 cc. of sesame oil at each dose in order further to saturate the body with the hormone. The animals stood the increased dose well.

Forty-two days after receiving the virulent bacilli another tuberculin test was made. Each animal reacted well to 5 mg. of O.T. and there was no significant difference between the different groups.

Whenever an animal in the group receiving the hormone died, an animal from each of the control groups was killed for comparison of the extent of the lesions. All animals that had not died were killed and autopsied 44 days

TABLE 1

Days after virulent infection	Microscopic Tuberculosis															
	Immunized progynon-B				Immunized sesame oil control				Immunized saline control				Non-immunized saline control			
	Spleen	Bronchial nodes	Lung	Liver	Spleen	Bronchial nodes	Lung	Liver	Spleen	Bronchial nodes	Lung	Liver	Spleen	Bronchial nodes	Lung	Liver
26	o	o	o	□	1+	1+	□	2+	1+	□	□	1+	5+	4+	1+	2+
29	—	—	—	—	—	—	—	—	—	—	—	—	4+	3+	1+	3+
30	o	□	o	o	1+	□	□	□	□	o	o	□	3+	3+	□	3+
34	□	o	o	o	1+	□	o	□	□	1+	o	1+	4+	4+	1+	2+
44	1+	o	1+	□	□	o	o	□	□	o	□	□	4+	3+	o	3+
44	1+	□	□	o	□	o	o	o	1+	o	o	o	5+	5+	3+	4+
44	o	o	o	o	o	□	1+	o	□	□	□	□	4+	4+	1+	3+
44	o	o	o	o	1+	o	o	□	□	□	o	1+	4+	3+	□	3+
44	1+	1+	□	o	1+	2+	□	1+	1+	1+	o	1+	4+	4+	3+	4+
44	1+	□	□	o	1+	2+	o	□	o	o	o	□	3+	3+	o	3+
44	1+	o	o	o	1+	□	o	o	o	1+	o	o	4+	—	2+	3+
44	3+	□	o	□	o	2+	□	1+	□	o	□	o	5+	3+	1+	3+
44	1+	o	o	o	o	o	o	□	—	—	—	—	—	—	—	—
44	□	1+	o	□	—	1	—	—	—	—	—	—	—	—	—	—

after receiving the virulent tubercle bacilli. In the group that received the hormone the uteri of all animals showed the macroscopic and microscopic alterations characteristic of hyperestrinism.

As shown in table 1, all of the animals in the control, non-immune group developed extensive macroscopic tuberculosis, whereas the immunized animals were markedly protected. In the latter, macroscopic lesions were virtually absent, and the lesions found microscopically were few and small. It is quite likely that some of the rare minute tubercles found in the spleen and bronchial nodes of the immunized animals were the result of the attenuated bacilli with which the animals were immunized, rather than a spread of the virulent infection. In table 1, even a single minute microscopic tubercle in an organ is listed as 1+.

The table shows that there was no significant difference between the amount of tuberculosis in the immunized animals that were kept continually in estrus and those that received the control solutions of sesame oil diluent or saline. The slight differences shown are well within the limits of variation to be expected in any series of animals given the same dose of bacilli. The con-

tinual flooding of the body with estrogenic hormone, had, therefore, no demonstrable effect upon acquired resistance to the tubercle bacillus.

SUMMARY

Under the conditions of these experiments, a marked degree of hyperestrinism exerted no detectable effect upon either native or acquired immunity to tuberculosis in the virgin female guinea pig. This suggests that some factor other than the premenstrual rise in estrogenic hormone is responsible for the premenstrual exacerbations of the disease that have been described in tuberculous women.

This work was instigated by Dr. Arnold R. Rich, and to him we are greatly indebted.

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THE THYROTROPIC HORMONE IN THE PITUITARY OF THE ALBINO RAT DURING GROWTH, PREGNANCY AND LACTATION[1]

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WITH THE DEVELOPMENT of a sensitive assay method for the thyrotropic hormone using the day-old White Leghorn chick (1), it became possible to determine the amount of this factor present in the pituitary of small experimental animals during growth, reproduction and lactation. The studies of Junkman and Schoeller (2) and McQueen-Williams (3) indicated that the rat pituitary contained relatively large amounts of this factor, so this animal was chosen for the initial study. As the lactogenic hormone of the rat pituitary had been traced through the same stages (4) the relation of these two hormones to growth and milk secretion was an added reason for study of the rat.

It is recognized that the amount of hormone present in the pituitary at any given stage of development may not represent the rate of its secretion, yet until such data has been obtained it will not be possible to determine the true situation.

The most extensive study of the thyrotropic hormone in other species, is that reported by Reece and Turner (4) in cattle. The results of this study will be discussed later in comparison with the present observations. Saxton and Loeb (5) have assayed human pituitaries for the thyrotropic hormone. No significant changes were observed with age, pregnancy or lactation.

EXPERIMENTAL

Procedure. Normal albino rats in various stages of growth and senescence were sacrificed and their pituitaries removed and weighed to the nearest .05 mg. on a precision balance. The animals were grouped as to body weight by 50 gm. intervals. Groups of both male and female animals were obtained. After sexual maturity of the females was attained, they were uniformly killed in a diestrous condition. For comparison, a group of estrous rats was obtained. During pregnancy 4 groups were obtained at 5, 12, 16, and 21 days and during lactation, 5, 10, 16 and 21 days (table 1).

Following collection of the pituitaries, they were frozen and held in that condition until ready for assay. The pituitaries in each group were prepared for assay by maceration and suspension in distilled water. The details of the assay method (1) using day-old White Leghorn chicks were carefully followed. With one exception, all

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chicks were injected with the same dosage of pituitary suspension (3 mg. total dosage). From the average weight of the chick thyroids, the chick units of thyrotropic hormone were computed by the equation presented by Bergman and Turner (1).

Observations. It is interesting to note that both sexes show a distinct rise in the concentration of the thyrotropic factor during the period of rapid growth (table 1). When the growth rate slows down and fattening normally occurs, the thyrotropic hormone potency decreased. While the ages were not known, the heaviest rats of both sexes were usually older animals. It is

TABLE 1. THE THYROTROPIC HORMONE CONTENT OF THE RAT PITUITARY

No. of rats in group	Condition and sex	Rat body wt. average gm.	Rat pituitary weight average mg.	Weight pituitary injected per chick mg.	Ave. wt. of chicks gm.	No. of chicks in group	Sex of chicks	Chick[1] thyroid weight average mg.	Standard error of mean	Chick units per gland	Chick[2] units per gm. of pituitary	Chick units per 100 gm. body wt. rat
62	♂	75.9	3.19	3	54.1	21	♂	4.85	±0.271	0.451	141.0	.594
23	♂	129.0	4.58	3	57.8	19	♂	5.51	±0.283	1.523	332.8	1.280
72	♂	174.0	5.46	3	55.0	61	♂	5.60	±0.254	1.888	345.9	1.084
31	♂	224.7	8.13	3	53.0	25	♂	4.68	±0.203	1.807	210.0	.813
40	♂	274.9	9.14	3	55.0	44	♂	4.44	±0.227	1.593	174.0	.580
12	♂	327.4	10.39	3	57.0	20	♂	4.73	±0.348	2.258	217.0	.689
11	♂	372.7	8.84	3	50.4	18	♂	4.60	±0.260	1.750	198.0	.669
46	Normal ♀	75.47	3.906	3	55.6	19	♂	4.08	±0.395	.473	121.0	.626
42	♀	123.60	6.004	3	55.8	22	♂	4.67	±0.275	1.250	208.0	1.014
28	♀	170.00	9.020	3	53.4	37	♂	3.91	±0.194	.869	96.0	.511
10	♀	226.00	10.88	3	51.1	21	♂	4.12	±0.180	1.385	127.0	.601
7	♀	259.00	13.89	3	54.9	19	♂	4.20	±0.295	1.404	139.0	.542
16	Estrus ♀	191.00	10.08	3	51.4	18	♂	3.606	±0.143	.507	50.0	.261
5	5 day preg. ♀	169.4	8.20	3	51.3	12	♀	4.94	±0.278	.445	54.0	.263
7	12 ♀	227.0	10.45	3	49.3	20	♀	4.47	±0.439	—	—	—
11	16 ♀	192.0	8.92	3	52.7	22	♀	5.33	±0.391	1.433	160.0	.751
12	21 ♀	254.5	11.14	3	51.0	20	♀	5.22	±0.395	1.556	130.0	.672
6	5 day lact. ♀	202.6	9.41	3	55.4	17	♂	4.66	±0.185	1.948	207.0	.970
8	10 ♀	206.2	11.20	3	52.5	20	♂	4.41	±0.281	1.904	170.0	.923
5	16 ♀	212.6	8.86	3	47.3	13	♂	4.80	±0.255	2.003	229.0	.941
12	21 ♀	236.5	11.05	3	52.7	20	♂	4.67	±0.465	2.402	208	1.013

[1] Average chick thyroid weights are total weights of paired glands.
[2] Chick units are based on equations: male, $x = .4439y - 1.443 \pm .126$
female $x = .8199y - 3.883 \pm .146$.

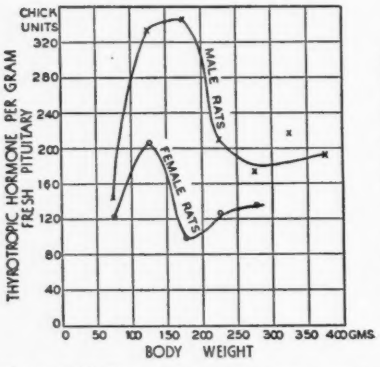
of interest to note that these animals did not show further decline in potency (fig. 1).

On the basis of body weight, the pituitaries from females weighing an average of 259 gm. and males weighing an average of 275 gm. contained about 50% as much hormone as did pituitaries from males and females weighing 129 and 124 gm. respectively.

The distinct difference in the concentration of the thyrotropic hormone in the male and female pituitary is significant. In every weight group, the male pituitary was higher, the female containing an average of 79% as much as the male per gland. In this connection it should be noted that the female pituitary is somewhat heavier than the male. On the basis of concentration of thyrotropic hormone per gram of fresh tissue, the female pituitaries contained only 62% as much as the males. May this difference explain in part the more rapid and extended growth of the male rat?

The change in the pituitary thyrotropic hormone during reproduction and lactation is of especial interest to us. The assay on the estrous rats was very low, with little change during the first half of pregnancy. The groups of animals pregnant 16 days and 21 days (just before parturition) showed definite increases in the amount of hormone (fig. 2).

Following parturition and the initiation of lactation there was a further increase in the amount of thyrotropic hormone present averaging 40% more



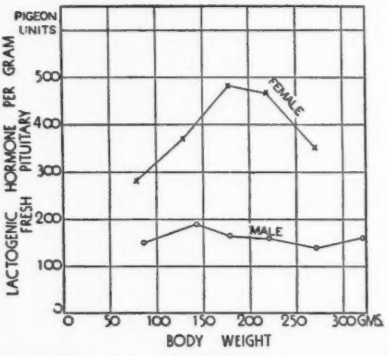


Fig. 1. (left) THE THYROTROPIC HORMONE CONTENT OF THE PITUITARY OF THE RAT DURING GROWTH
Fig. 2. (right) THE LACTOGENIC HORMONE CONTENT OF THE RAT PITUITARY DURING GROWTH (Reece and Turner-1937).

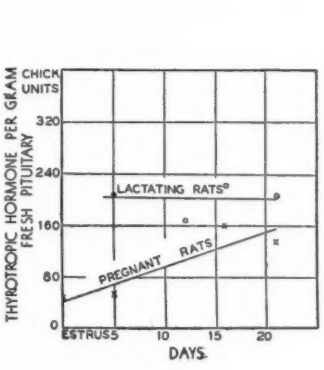
than during the last two stages of pregnancy. No significant trend in the concentration during lactation was noted.

DISCUSSION

To understand the rôle of the pituitary hormones during growth, reproduction and lactation it will be necessary to trace the rate of secretion of each hormone during these interesting sexual epochs. To obtain this information, cytologists have studied the pituitary cell types for evidence of cellular secretion and storage (6). A more direct approach to the problem would result from a correlation of the hormone content of the pituitary, the blood and the urine. If it could be shown that high pituitary potency is related to high blood and/or urine concentration, definite proof would be afforded. In the absence of such data other evidence of the relation of pituitary content to secretory activity must be sought.

In a previous study of the lactogenic hormone of the rat pituitary (4), the amounts present during various stages were such as would be expected if the amount present in the pituitary represented the rate of secretion of the hormone. The amount of lactogen in the male rat pituitary remained low throughout the life of the animal (fig. 3). In the female, the amount of hormone increased with puberty (probably under the stimulus of estrogen) and remained at about the same level during pregnancy. Following parturition, however, there was a very marked increase in the amount present, which

would be expected on the basis of the activity of the mammary glands at that time (fig. 4). It is true that there seems to be a storage of lactogen during the interval between nursings and a release following the stimulus of nursing but in the natural condition the cycles of secretion, storage and discharge are of such short duration that pituitaries obtained at any time are indicative of the rate of lactogen secretion. There is nothing in this work to indicate that the lactogenic hormone content of the pituitary may not be considered as representing the rate of lactogen secretion at that time.



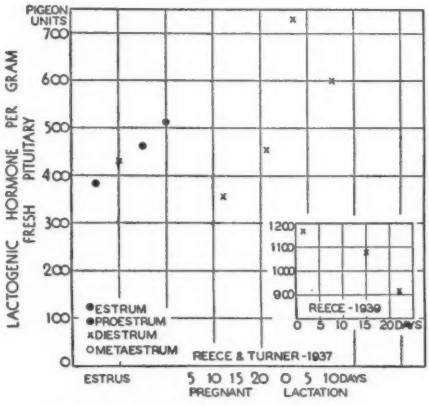


Fig. 3. (left) THE THYROTROPIC HORMONE CONTENT OF THE PITUITARY OF THE RAT DURING PREGNANCY AND LACTATION. Fig. 4. (right) THE LACTOGENIC HORMONE CONTENT OF THE PITUITARY OF THE RAT DURING ESTRUS, PREGNANCY AND LACTATION (Reece and Turner-1937, Reece et al.-1939).

The present data if interpreted as representing the rate of thyrotropic hormone secretion would indicate rapid secretion during the period of rapid growth. It is known that the secretion of the thyroid gland is essential for growth. Rats thyro-parathyroidectomized at birth do not grow even when given pituitary implants (7). Later, the rate of growth is seriously impaired. It seems reasonable to expect that the thyrotropic hormone should be secreted in large amount when growth is very rapid. The sex difference in the thyrotropic hormone is also of great interest. May not this be related to the difference in the rate of growth of the two sexes? In the assay of the growth hormone it is significant that the female is more responsive than the male and further the best 'growth hormone' preparations still contain considerable amounts of the thyrotropic factor (8).

In comparison, the data on the thyrotropic hormone content of cattle pituitary is of interest (4). The sex comparisons are similar to those of the rat, young bulls contained 19% more hormone than heifers per unit weight of fresh anterior lobe tissue whereas in older animals the difference was 42% in favor of the bulls. During growth, the thyrotropic concentration in the pituitary gland increased until the animals reached 4 to 10 months, after which it decreased. In relation to potential lactation, it was noted that in

all comparisons, dairy cattle pituitaries contained considerable more thyrotropic hormone than beef cattle.

The data now available on the lactogenic and thyrotropic hormones present during growth may be considered in relation to the synergism postulated by Riddle and associates (9, 10). In the male rat, where growth is more rapid and more extended, the lactogenic hormone remains low but the thyrotropic hormone is high. In the female, the lactogenic hormone increases with puberty while the thyrotropic hormone remains definitely lower than in the male. It would appear that of these two hormones, thyrotropic is the more important in relation to growth, if indeed, the lactogenic has any function at all in connection with growth. In fact, it is the opinion of the writers that the lactogenic hormone has but one primary function in mammals, namely, to initiate and assist in maintaining secretory activity of the epithelial cells of the mammary gland.

The distinct rise in the amount of the thyrotropic hormone in the pituitary following parturition is believed to be of considerable interest. It is known that milk secretion rises for a time, then declines. The mechanism regulating the lactation curve has long been sought. It would appear that the increased rate of secretion of both lactogenic and thyrotropic hormones after parturition influences the rising segment of the curve. Evidence for the influence of thyroxine on lactation has been reviewed (11).

The significance of these data in relation to the inheritance of milk yield may be pointed out. It has been suggested by the senior author (12) that while the inherited variation in the productivity of dairy cattle may be in part of a general nature, the physiological expression is due in large part to the interaction of the hormones of the pituitary and the other endocrine glands in stimulating the growth of the mammary glands (with mammogen), in stimulating the secretory epithelium of the gland (with lactogen), in stimulating general metabolism (with thyrotropin), in stimulating the adrenal cortex (with adrenocorticotropic factor) and in stimulating the metabolism of the various precursors of milk in the blood by the carbohydrate, fat and protein metabolism hormones.

Marked increases in the rate of secretion of the lactogenic and thyrotropic hormones following parturition during the period of intense lactation would indicate their functional importance. That this influence is quantitative is indicated by the increase in lactation in dairy cattle following the injection of various pituitary hormones as well as thyroxine.

SUMMARY

A study is reported on the thyrotropic hormone content of the pituitaries of normal male and female albino rats during various stages of growth, reproduction and lactation.

The pituitaries were assayed in groups using day-old White Leghorn chicks. The Bergman-Turner chick units of hormone were determined from the average weight of the chick thyroids by means of a suitable equation.

The concentration of the thyrotropic factor in the pituitary of both sexes

increased during the period of rapid growth. However, the females contained only about 50% as much hormone as the males during this period. In a comparison of all weight groups, it was observed that the female pituitaries contained 62% as much as the male per gram of fresh tissue.

In the estrous female the hormone concentration was low and continued so during the first half of pregnancy. There was evidence of a definite increase in hormone concentration during the latter part of pregnancy and a further 40% increase during lactation.

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A COMPARISON OF THE GUINEA PIG AND CHICK THYROID IN THE ASSAY OF THE THYROTROPIC HORMONE[1,2]

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ABOUT 10 years have passed since Loeb and Bassett (1) reported that injections of extracts of the bovine pituitary produced marked hypertrophy of the guinea pig thyroid. This observation was soon confirmed by Aron (2). In a comparison of several species, Thurston (3) observed that the sensitivity of the thyroid gland to thyrotropic hormone was low in the mouse and rat but increased in the rabbit, cat, pigeon and guinea pig. Most investigators have suggested the use of the immature guinea pig in the biological assay of this hormone (4–7, 9–13) although Collip and Anderson (8) have used the hypophysectomized rat and Smelser (14, 15) the day-old chick. As a measure of activity, histological observation of the thyroid gland was employed most frequently in the earlier papers (4, 5, 6, 7, 9, 12) while weight increase of the thyroid is gradually becoming more popular (10, 11, 14, 15, 16). Metabolic activity (8, 9) and change in the iodine content of the thyroid (13) have also been suggested. For a recent review of assay methods, the reader is referred to the papers by Rawson and Starr (7).

In connection with our study of the chemical separation of the pituitary hormones and the assay of various fractions for thyrotropic activity, the weight increase of the immature male guinea pig thyroid has been used with satisfactory results. However, a more sensitive method was sought which would make possible a study of changes in the thyrotropic hormone content of the pituitary of experimental animals during growth, sexual cycles, pregnancy, lactation and involution.

Dr. E. T. Gomez working in this laboratory observed that day-old White Leghorn chicks showed a marked increase in thyroid weight upon the injection of certain extracts. Preliminary studies with the pituitaries of rabbits and rats indicated that the chick was sensitive to this hormone. While these studies were in progress, the preliminary report on the use of the chick in the bioassay of this hormone was made by Smelser (14). Our observations, in general, confirm his findings although there are a number of points of dif ference with Smelser's more recent paper which will be discussed later (15).

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 593.

The object of the present report is to present our extensive experience in the use of the guinea pig and day-old chick in the bioassay of the thyrotropic hormone, their relative sensitivity, repeatability of assay, sex differences in the chicks, effect of increasing dosage, and finally to suggest a guinea pig and chick unit of hormone.

EXPERIMENTAL METHODS

Preparation of thyrotropic hormone. The pituitary preparation used in the experiments reported in this paper is the initial extract of cattle pituitaries, the preparation of which was described by Bergman and Turner (17). This extract is known to contain large percentages of the lactogenic, carbohydrate metabolism, gonadotropic and thyrotropic hormones of the original pituitaries. For the purpose of injection, the dried preparation was dissolved in a slightly alkaline solution (with NaOH), then precipitated by bringing the solution to the isoelectric point with HCl. The volume of fluid injected daily amounted to 0.1 cc. in the chick and 0.25 cc. in the guinea pig.

Source of animals. The chicks used were White Leghorns separated as to sex obtained from a commercial hatchery. They were received on the day following hatching, placed in cages heated with electric light bulbs in an automatic temperature controlled room (75 to 80° F.). They were fed a regular growing mash and provided with ample water. Due to the variation in body weight, the chicks were usually divided into two groups, under and over 38 gm. In the heavier group, injections were begun on the day of arrival, while in the lighter group, the first injection was delayed a day. This practice produced a more uniform body weight at the end of the injection period.

Male guinea pigs weighing between 120 and 150 gm. were obtained from commercial breeders. On arrival the animals were placed in an animal room with automatic temperature control at 75° to 80° F. and kept for several days before injections were started. They were fed a mixture of grain feed and lettuce.

Experimental procedure with chicks. The chicks were given 4 daily subcutaneous injections and killed 24 hours after the last injection. The thyroids were dissected with the aid of a binocular loupe and immediately weighed on a precision balance. At the end of the injection period, only chicks weighing from 45 to 65 gm. were included. It is desirable that the mean weight should be about 55 gm.

The thyroid weights of normal uninjected control chicks of both sexes, killed on the fifth day (sixth day after hatching) have been obtained at intervals throughout the experiment. To determine the influence of increasing dosage of the initial extract, groups of 20 chicks of each sex were injected with increasing amounts ranging from 0.2 to 4 mg. over a period of 4 days. In any group of 20 chicks, only 4 to 8 were injected at one time, thereby insuring chicks from at least 3 to 5 different hatchings.

It will be observed that the thyroid weights of the normal male and female chicks (controls) vary considerably (table 1), the mean thyroid weight of males being 1.30 mg. less than that of the females. Additional groups of normal male and female chicks obtained in the course of routine assays were quite similar to the above, 28 male thyroids having a mean of 3.7 mg. and 36 females a mean of 4.8 mg.

Upon the injection of increasing amounts of extract up to 1.0 mg. in 4 days there was a regular increase in thyroid weight. Upon doubling or quadrupling the dosage relatively little increase was noted. From these data, it is clear that the most sensitive range for the assay of this hormone

is obtained when the thyroid weight is less than 8 mg. As the relation between thyroid weight and dosage was essentially linear in nature up to 1.0 mg. regression equations of the type $y=a+bx$ were fitted to the data (fig. 1). The regression equations were based on correlation coefficients between thyroid weight and injection dosages up to 1 mg. of 0.969 ± 0.042 for male

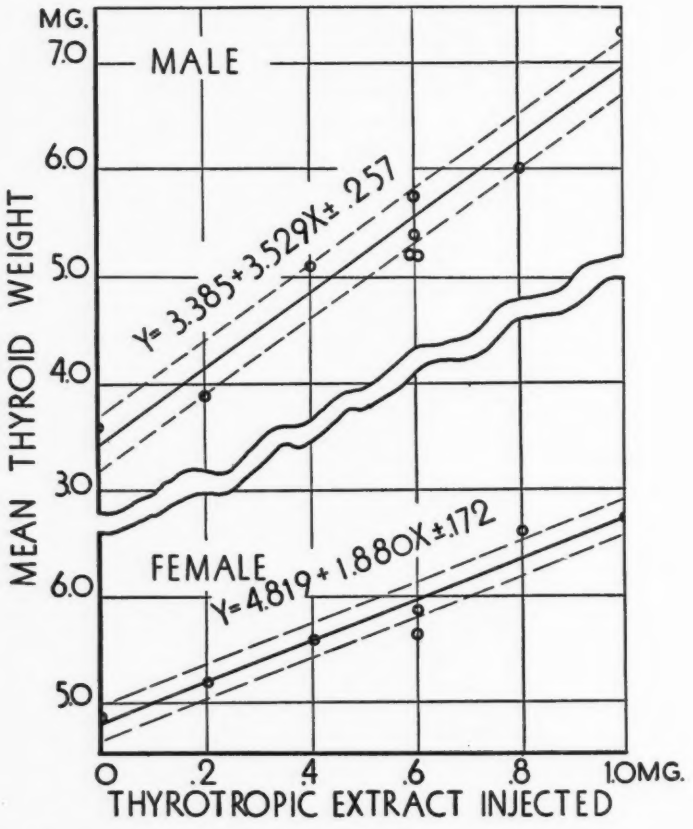

Fig. 1. The relation between increasing amounts of thyrotropic extract and mean thyroid weight of male and female chicks. From the regression equations, it will be observed that the mean thyroid weight of the males increases twice as rapidly as the females. The broken lines indicate the standard error of estimate of the regression equations.

chicks and 0.960 ± 0.020 for female chicks as calculated by Pearson's product movement method of simple correlation. The data indicate distinct differences in the normal thyroid weight of male and female chicks, 3.39 mg. for males, 4.82 for females. Even more important is the difference in the slope of the two regression lines. For a given amount of thyrotropic hormone injected, the equation shows that the male thyroid weight increases almost

twice as rapidly as the female. In other words, the male chick is twice as sensitive as the female chick to thyrotropic hormone. Due to the difference in control weight as well as the sensitivity of response of male and female chicks, it is preferable to separate the two sexes unless equal numbers are included in each assay group.

As sexed male chicks are cheap and readily available, it seemed desirable to establish an assay unit in terms of this sex. From a study of our data it appeared that a 50% increase in thyroid weight would be significant and yet would be within the most sensitive range.

A male chick unit (ch.u) of thyrotropic hormone is tentatively defined as the total amount of hormone administered over a 4-day period, with sub-

TABLE 1. EFFECT OF THYROTROPIC HORMONE ON CHICK THYROID

Thyrotropic Hormone mg.	Male				Female			
	No. of Chicks in Group	Body Wt. Mean gm.	Thyroid Wt. Mean mg.	Range Min. Max.	No. of Chicks in Group	Body Wt. Mean gm.	Thyroid Wt. Mean mg.	Range Min. Max.
Control	20	55.3	3.6±.16[1]	2.4- 5.5	30	54.6	4.9± .43[1]	2.7- 9.8
0.2	20	55.0	3.9±.26	2.5- 5.9	20	54.7	5.2± .43	2.7- 9.8
0.4	20	54.7	5.1±.33	3.1- 8.4	20	55.4	5.6± .43	3.5-10.5
0.6	20	54.4	5.4±.50	2.9-11.8	20	54.7	5.9± .54	3.2-14.2
0.8	20	54.8	6.0±.33	3.4- 8.8	20	55.1	6.6± .73	3.1-16.4
1.0	20	54.2	7.3±.77	3.0-16.7	20	54.8	6.7± .56	3.3-15.6
2.0	20	54.8	7.9±.58	3.9-13.1	20	54.0	7.0± .56	3.6-12.2
4.0	20	54.1	7.6±.50	3.5-14.8	20	54.9	10.2±1.17	4.4-22.6

[1] Standard Error of Mean $= \frac{\text{S.D.}}{\sqrt{N}}$.

cutaneous injections once each day, which will cause a mean weight increase of 50% (to about 5.4 ± 0.26 mg.) in the thyroids of 20 chicks weighing an average of 55 ± 10 grams.

On the basis of this definition, one chick unit (male) of thyrotropic hormone is contained in 0.6 mg. of the initial extract. To determine the reproducibility of the assay method, 3 additional groups of 20 chicks each were injected at the 0.6 mg. level with the following results:

Group 2	54.8 gm. body weight	5.2 ± 0.30 mg. thyroid weight
Group 3	54.1 gm. body weight	5.7 ± 0.51 mg. thyroid weight
Group 4	53.3 gm. body weight	5.2 ± 0.28 mg. thyroid weight

The mean thyroid weight of 80 chicks injected with 0.6 mg. was 5.38 mg. It will be seen that all 4 groups are practically within the limits of the standard error of estimate of the regression equation. On the average, 2 out of 3 assay groups should come within these limits.

In formulating a ch.u. of hormone based upon female chicks, two courses are open. One could demand a 50% increase in the thyroids above the control weight in which case the amount of hormone indicated by the two units would be quite different or define the female chick unit in terms of approximately equivalent amounts of hormone. We prefer the latter method for the

reason that a 50% increase would demand a relatively large amount of hormone and further would approach a region of lessened sensitivity.

At the 0.6 mg. injection level the mean thyroid weight of the female chick was 5.9 mg. (regression equation value = 5.95 mg.). This is an increase of slightly more than 20% in thyroid weight above the controls. The female chick unit would be tentatively defined as the total amount of hormone administered over a 4-day period, with subcutaneous injection once each day, which will cause a mean weight increase of 20% (to about 5.9 ± 0.17 mg.) in the thyroids of 20 chicks weighing an average of 55 ± 10 grams.

Where the amount of test material is limited, it is frequently desirable to estimate the units of thyrotropic hormone from the mean thyroid weight obtained. The units of thyrotropic hormone in an unknown preparation when either male or female chicks are used can be determined from the regression equation $x = a + by$, where x represents the units of thyrotropic hormone and y the mean weight of the thyroids (table 2). It is preferable in precise assay work to obtain thyroid weight closely approximating a unit response.

TABLE 2. RELATION OF EXPERIMENTALLY PRODUCED THYROID WEIGHT AND CHICK UNITS

Thyroid Weight of Chicks Mean mg.	Chick Units of Thyrotropic Hormone Male[1]	Chick Units of Thyrotropic Hormone Female[2]
3.5	—	—
4.0	0.33 ± 0.126	—
4.5	0.55 ± 0.126	—
5.0	0.77 ± 0.126	0.21 ± 0.146
5.5	0.99 ± 0.126	0.62 ± 0.146
6.0	1.21 ± 0.126	1.03 ± 0.146
6.5	1.44 ± 0.126	1.44 ± 0.146
7.0	1.66 ± 0.126	1.85 ± 0.146

[1] Calculated from equation $x = 0.443y - 1.443 \pm 0.126$.
[2] Calculated from equation $x = 0.819y - 3.883 \pm 0.146$.

Experimental procedure with guinea pigs. From the results of earlier work the following standard procedure was followed. Male guinea pigs weighing between 140 and 170 gm. were injected subcutaneously once daily for 5 days and killed 24 hours after the last injection. The thyroids were dissected and trimmed with the aid of a binocular loupe, then weighed on a precision balance.

Control animals have been obtained periodically throughout the course of the experiment. These animals were injected with 5 mg. of a beef muscle extract. Three groups of 10 animals each are included. To determine the influence of increasing amounts of the initial extract upon the thyroid weight of the guinea pig, groups of 10 animals were injected with amounts varying from 0.5 to 10 mg. In these groups not more than 2 to 4 animals were injected at any one time so that from 3 to 5 different shipments of guinea pigs covering some time interval were included.

With the increase in the amount of extract injected there was a gradual increase in the average weight of the guinea pig thyroid (table 3). As the points appear to be distributed in a fairly linear manner, a regression equa-

tion was fitted to the data. The fit to the data is quite satisfactory except for the control groups which appear slightly low (fig. 2). If the actual control data are used, a 50% increase in the thyroid weight would be about 26.4 mg. whereas if the calculated control is used a 50% increase would bring the weight demanded up to about 29.9 mg. As this would require twice as much hormone it seems preferable to define a unit in terms of the actual

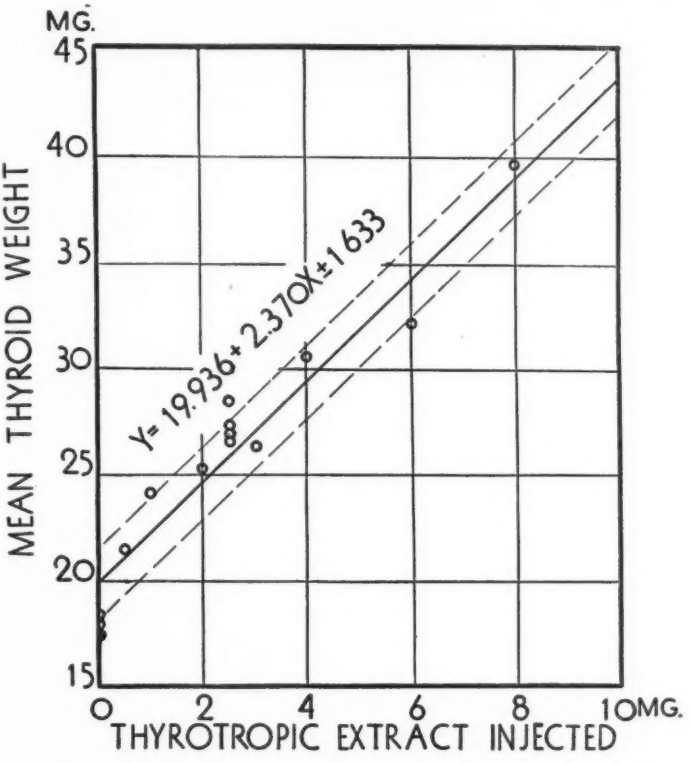

Fig. 2. The relation between increasing amounts of thyrotropic extract and mean thyroid weight of male guinea pigs. The broken lines indicate the standard error of estimate of the regression equations.

control data. A guinea pig unit (G.P.U.) of thyrotropic hormone is tentatively defined as the total amount of hormone administered over a 5-day period, with subcutaneous injection once each day which will cause a mean weight increase of 50% (to about 26.4 ± 1.63 mg.) in the thyroids of 10 male guinea pigs weighing an average of 155 ± 15 gm.

Since 2.5 mg. of extract elicited a mean thyroid weight of 27.4 mg. which is within the range of increase specified, it was decided to determine if reproducible results could be obtained at the same level of injection. There-

TABLE 3. EFFECT OF THYROTROPIC HORMONE ON MALE GUINEA PIG THYROID

Thyrotropic Hormone mg.	Male				
	No. of Guinea Pigs in Group	Body Wt. Mean gm.	Thyroid Wt. Mean mg.	Ratio Mean to Standard Error	Range Min. Max.
Control	10	153.6	17.7± .66[1]	26.8	15.0–21.5
Control	10	156.9	17.9± .84	21.3	14.3–22.5
Control	10	156.7	17.2± .79	21.8	13.4–21.4
0.5	10	155.3	21.6±1.53	14.1	14.2–29.3
1.0	10	155.9	24.2±1.11	21.8	18.1–28.8
2.0	10	156.5	25.3±1.28	19.8	17.9–30.0
2.5	10	155.2	27.4±1.17	23.4	22.5–33.3
3.0	10	156.3	26.4±1.25	21.1	21.9–32.9
4.0	10	157.8	30.6±0.82	37.3	25.9–34.4
6.0	10	154.3	32.2±1.67	19.3	26.1–43.2
8.0	10	155.7	39.7±1.05	37.8	34.2–45.3
10.0	10	156.9	41.9±1.84	22.8	31.1–50.0

[1] Standard Error of Mean $= \frac{\text{S.D.}}{\sqrt{N}}$.

fore 3 groups of 10 male guinea pigs were injected with 2.5 mg. with the following results:

Group 2 155.1 gm. body weight 27.1 ± 1.32 mg. thyroid weight
Group 3 156.1 gm. body weight 28.6 ± 1.25 mg. thyroid weight
Group 4 157.4 gm. body weight 26.6 ± 1.41 mg. thyroid weight

The mean thyroid weight of these 40 guinea pigs was 27.4 mg. which is the average of the first group.

It is frequently desirable to estimate the units of thyrotropic hormone from the mean thyroid weights of the male guinea pig. As with chicks a regression equation of the type $x=a+by$ has been calculated (table 4). Here, too, it is preferable to obtain thyroid weights approximating a unit response.

TABLE 4. RELATION OF EXPERIMENTALLY PRODUCED THYROID WEIGHT AND GUINEA PIG UNITS

Thyroid Weight of Guinea Pigs Mean mg.	Guinea Pig Units of Thyrotropic Hormone[1]
22	0.40±0.268
24	0.72±0.268
26	1.04±0.268
28	1.36±0.268
30	1.68±0.268
32	2.00±0.268

[1] Calculated from equation $x=0.160y-3.116\pm0.268$.

DISCUSSION

Most rapid progress in endocrine research depends upon the development of suitable assay methods for the various hormones. For use in connection with studies of the chemical separation of the pituitary hormones, reproducibility of bioassay is of paramount importance. The amount of hormone required is only secondary. For study of the variation in the thyrotropic hormone in the pituitary of experimental animals, the most sensitive

assay animal available should be used as only minute amounts of hormone are present. Accuracy as measured by reproducibility of assays is of no less importance.

As here defined, the male chick unit of thyrotropic hormone was found to be about ¼ the amount of the guinea pig unit.[3] In this respect our observations differ from the conclusions of Smelser (16) who reported chick responses to one-tenth the requirement of the guinea pig. We also found great differences in the thyroid weight of the male and female chicks as well as sensitivity to the thyrotropic hormone. It would seem inadvisable to use male and female chicks indiscriminately due to these differences. It is suggested that either one or the other sex be used unless equal numbers are included in each group. As the thyroid weight of the male chick increases about twice as fast as the female following the injection of increasing amounts of the thyrotropic hormone, we prefer to use the male chick. The chicks used in this study appear to vary more widely in thyroid response than did Smelser's.

The number of animals required for an assay varies with the reproducibility desired. In homogenous stock small numbers may be satisfactory. It appears that the chicks used in the present study, although coming from a single hatchery, were more variable in thyroid weight than those used by Smelser. As a test of reliability, the ratio of the mean to its standard error may be used. By this test it was found that the ratios for groups of 10 guinea pigs were distinctly higher than the ratios for groups of either 10 male or female chicks. When the groups of chicks were increased to 20, the ratios for the guinea pigs were still higher than the chicks (tables 1 and 3).

The rate of response to hormone administration usually declines as the amount injected increases. It is generally conceded that the initial part of the curve is most sensitive as in this region there is frequently a straight line relationship between dose and response. In both the male chick and guinea pig, this relationship holds true considerably beyond the 50% increase weight range. While it is desirable to obtain weight responses in this region of the curve, it would appear that one is justified in calculating the unitage at somewhat lower or higher thyroid weights in either chick or guinea pig. As an aid in the calculation of chick or guinea pig thyrotropic units, regression equations have been computed.

In our judgment, where the amount of hormone, cost, or availability of animals is not a problem, we prefer to use 10 guinea pigs rather than 20 male chicks in the assay of the thyrotropic factor. If chicks are to be used, the male chick is to be preferred to the female. The advantages of the male chick is the general availability throughout the year in any number desired, low cost in comparison to the female chick or guinea pig and finally the lower requirement of hormone for an assay.

SUMMARY

A study is reported concerning the relative value of the immature male guinea pig and day-old male and female White Leghorn chicks in the assay of the thyrotropic hormone of the anterior pituitary.

[3] In this connection it should be appreciated that the guinea pig weighs about 3 times as much as the chick. On an equal body weight basis, the male chick is only slightly more sensitive to the thyrotropic hormone than the guinea pig.

Groups of 10 guinea pigs and 20 male and female chicks were injected with increasing amounts of the initial extract of the AP. In the case of the chicks it was noted that the mean normal thyroid weight of the males was distinctly lower than the mean weight of the females. Further the regression equations calculated for the experimental observations showed that the slopes of the lines were twice as great for the males as for the females indicating greater sensitivity in response to the thyrotropic hormone in the former.

From these data a male chick unit of thyrotropic hormone is tentatively defined as the total amount of hormone, administered over a 4-day period, with subcutaneous injection once each day which will cause a mean weight increase of 50% (to about 5.4 ± 0.26 mg.) in the thyroids of 20 chicks weighing an average of 55 ± 10 gm. In order to define the female chick unit in terms of approximately equivalent amounts of hormone it is defined as the total amount of hormone administered over a 4-day period, with subcutaneous injection once each day which will cause a mean weight increase of 20% (to about 5.9 ± 0.17 mg.) in the thyroids of 20 chicks weighing an average of 55 ± 10 gm.

The guinea pig unit of thyrotropic hormone is tentatively defined as the total amount of hormone administered over a 5-day period, with subcutaneous injection once each day which will cause a mean weight increase of 50% (to about 26.4 ± 1.63 mg.) in the thyroids of 10 male guinea pigs weighing an average of 155 ± 15 gm.

Three additional groups of male chicks and guinea pigs were injected at the unit dosage to determine the reproducibility of the assay methods. In both cases the results were considered satisfactory.

As here defined, the male chick unit of thyrotropic hormone was found to be about ¼ the amount of the guinea pig unit. On an equal body weight basis, the male chick is only slightly more sensitive than the guinea pig.

For the determination of the units of thyrotropic hormone in unknown preparations, regression equations of the form $x = a + by$ where x represents units of hormone and y the mean weight of the thyroids have been calculated.

We wish to express our sincere thanks to Mr. John P. Mixner for his assistance with the statistical analysis of the data.

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A STUDY OF THYROTROPIC HORMONE IN CLINICAL STATES[1]

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In 1927 Smith (1) showed that the presence of the pituitary was necessary for the proper functioning of the thyroid gland in the rat. Since that time numerous workers have shown that suitable extracts of the anterior pituitary can produce hyperplasia of the thyroids in various laboratory animals: the guinea pig (2, 3), the dog (4, 5), the pigeon (6), the hen (7) the chick (8), the duck (9), the rat (10).

To date the only definite source of thyrotropic hormone has been the hypophysis, and the isolation of a thyrotropic substance from urine, plasma, or other tissues awaits proof. There have been numerous reports of the finding of thyrotropic substance in the urine of myxedematous patients (11, 12, 13, 14), and in hyperthyroid patients (15); in menopausal patients (16); and in normal urine and serum (11, 13) but other workers have been unable to confirm these findings (17, 18, 19, 20).

In most of the earlier work the urine or serum was injected into the test animal without any attempt to purify or concentrate the thyrotropic substance thought to be present, but Cope (20) claims to recover almost entirely a known amount of thyrotropic substance added to normal urine and extracted with benzoic acid.

The possibility of a normally occurring thyroid inhibitory substance in the blood was first suggested by Blum (21) and Anselmino and Hoffmann (22) described a lipoid fraction from normal blood and tissue which had similar properties. Scowen and Spence (23) described an inhibitory effect of normal rabbit serum when added to thyrotropic hormone and injected into guinea pigs. Further, normal patients exhibited a similar anti-thyrotropic effect, though patients with Graves disease showed none.

Collip and Anderson (24) described an anti-thyrotropic substance occurring spontaneously in the serum of certain patients with low B.M.R. Bodart and Fellinger (25) showed that an ether-soluble extract representing 150 cc. of blood from a normal patient inhibited the thyrotropic manifestations of an AP extract injected into guinea pigs. Less of this antagonism was found in hyperthyroidism and more in myxedema. Eitel and Loeser (26) found that serum of untreated sheep had an action antagonistic to thyro-

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tropic hormone. Thompson and Cushing (27) confirmed this finding in a single experiment when they found that lamb serum inhibited a substantial dose of sheep thyrotropic hormone, using guinea pigs as a test object.

The present study was undertaken with a view to: *a*), comparing the efficiency of various methods for the recovery of known amounts of thyrotropic substance from normal urine; *b*), attempting to recover thyrotropic substance from the urine of clinical cases which might theoretically be expected to excrete such a substance, that is myxedemas and acromagalics; *c*), attempting to demonstrate the presence of an anti-thyrotropic substance in the sera of a small group of patients. For the latter, schizophrenic patients were chosen because they frequently show a marked resistance to thyroid extract by mouth, amounts up to 60 gr. daily having been well tolerated. Further, it is rare if not completely unknown for a schizophrenic patient to suffer from thyrotoxicosis although Lewis (28) quotes two such cases reported in the German literature. Finally, the low basal metabolism frequently found in schizophrenia suggests a possible relationship between this clinical group and the cases described by Collip and Anderson (24) and Bodart and Fellinger (25) where a low B.M.R. accompanied an anti-thyrotropic activity of the serum.

EXPERIMENTAL

The chick[3] was used as the test object for the thyrotropic activity because as Smelser (8) has shown, it is more sensitive than the guinea pig, is cheaper, its thyroid has a more uniform histological structure and with moderate doses of thyrotropic substance it gives a relatively greater thyroid weight increase than the guinea pig.

The cytology and weights of the thyroids were studied, no attempt being made to determine blood iodine or basal metabolism, which offer alternative methods for assessing thyroid activity in the test animal. There are many objections to the use of the cytological test but these are well recognized and have led to much circumspection in the evaluation of results.

In the first place, the choice of a fixative is important and after some hesitation 10% formalin was adopted as the fixing solution for the glands. This was done because it was found that the acinar epithelium fixed by this method was much flatter than when fixed by Bouin or Zenker. For this reason a moderate degree of hyperplasia was much more readily detected than when the normal picture might show acini lined by high cuboidal epithelium. Such a consideration, though probably introducing an artefact, is nevertheless of real practical assistance in recognizing a mild degree of stimulation.

The normal chick gland fixed in this way gives a remarkably constant picture, the acini being lined by flat epithelial cells which contained a flat or oval nucleus and but little cytoplasm. The colloid was always abundant but frequently shrunken or broken, probably due to the fixative. In 2 of 46

[3] Smelser's work was repeated in part but as the results obtained were essentially the same, it was decided to use the chick rather than the guinea pig. Hypophysectomized rats and guinea pigs were also tried as possible test objects but the former were less sensitive than the chick, while the latter were unsuitable because of their high mortality rate after the operation.

untreated chicks, the cytoplasm was fairly abundant and the nucleus rounded, but in these glands the height of the cell was still less than its width. When the two diameters of many of the cells were equal (cuboidal epithelium) or when the epithelium became frankly columnar, then this was taken as indication of increased thyroid activity. For rough evaluation, the picture was considered plus when the majority of the acini were lined with columnar or cuboidal epithelium, plus or minus when some of the cells appeared to be slightly stimulated and showed something approaching cuboidal

TABLE 1. RESPONSE OF DAY-OLD CHICKS TO INCREASING DOSES OF THYROTROPIC HORMONE

Dosage	Number of Chicks	Thyroid				
		Weight Range	Mean Weight	Cytology[2]		
		mg.	mg.	−	±	+
4.0 U[1] in 3 days	10	3.5 to 6.0	4.8	6	4	0
8.0 U in 3 days	9	3.0 to 7.0	4.5	0	1	8
12.0 U in 3 days	9	3.0 to 9.2	5.3	1	2	6
16.0 U in 3 days	9	3.6 to 8.5	6.7	0	0	9
30.0 U in 3 days	5	5.5 to 7.4	6.6	0	0	5
60.0 U in 3 days	5	4.5 to 13.2	9.7	0	0	5
90.0 U in 3 days	5	6.5 to 11.2	8.5	0	0	5
0.5 U[1] in 5 days	4	3.0 to 3.5	3.4	4	0	0
1.0 U in 5 days	4	3.2 to 5.0	3.8	4	0	0
2.0 U in 5 days	9	2.0 to 6.5	4.0	3	3	3
4.0 U in 5 days	9	2.6 to 8.0	4.9	0	3	6
8.0 U in 5 days	8	3.0 to 7.2	5.5	0	0	8
12.0 U in 5 days	8	5.0 to 9.4	6.6	0	1	7

[1] Manufacturer's (Schering) evaluation.

[2] The thyroid epithelium was considered to be definitely stimulated (+) when the majority of the acini were lined with columnar or cuboidal epithelium, (±) when some of the cells appeared to be slightly stimulated and approached cuboidal epithelium, and (−) when the epithelium lining the glands was flat and ribbon-like.

epithelium, and minus when the epithelium lining the glands was flat and ribbon-like. Neither the colloid picture nor the weight of the glands was used as a criterion, although these were noted.

The thyrotropic hormone used was a powdered extract supplied by the Schering Corporation, the strength of which was given as 10 U per mg.

The chicks were White Leghorns which were killed when 4 to 7 days old. They were housed in roosts kept at a constant temperature by electric light bulbs from which the chicks were protected by wire netting.

Table 1 shows the response of the chick to various doses of Schering thyrotropic hormone given once daily, subcutaneously, for periods of 3 to 5 days. If one assumes that a cytological response in 2/3 of the animals used is evidence of thyrotropic activity, then it will be seen that 8 Schering U given in 3 days, and 4 Schering U given in 5 days, represent minimal effective doses.

Table 2 shows the effect of age on the thyroid of the untreated animal, there being no apparent change in the activity of the gland up to 9 days, but at 13 days there was definite hyperplasia and marked weight increase of the thyroids.

Knowing the minimal dose of Schering thyrotropic hormone required to produce activation of a chick thyroid, an attempt was now made to compare the efficiency of various methods for extracting this thyrotropic hormone added to normal urine obtained from medical students.

TABLE 2. EFFECT OF AGE ON THYROIDS OF UNTREATED CHICKS

Age When Killed	Number of Chicks	Thyroid				
		Weight Range	Mean Weight	Cytology		
		mg.	mg.	−	±	+
3 days	10	1.8 to 3.6	2.7	10	0	0
6 days	26	2.5 to 5.0	3.5	25	1	0
9 days	10	3.2 to 5.5	4.1	9	1	0
13 days	6	4.4 to 11.5	7.9	1	2	3

Table 3 shows the results with the 6 methods used. Actually the equivalent of 12 U was added for each chick used, the whole being dissolved in slightly alkaline solution and then added to 500 cc. of urine. The extract was then injected on 5 successive days. This dose (3 times the minimal effective dose) was chosen because at this level there is also a definite weight response of the chick thyroid.

The acetone method gave a definite response as judged both by the cytology and thyroid weight, and the activation produced is comparable with that caused by injecting directly 12 U of thyrotropic hormone for 5 days. The alcohol-ether method gave a definite cytological response without, however, giving as great a weight response as the acetone method. The benzoic acid method was the only other method used which appeared to recover any of the thyrotropic substance from the urine.

TABLE 3. EXTRACTION METHODS

Method	Number of Chicks	Thyroid				
		Weight Range	Mean Weight	Cytology		
		mg.	mg.	−	±	+
Acetone	5	4.6 to 9.6	7.1	1	0	4
Alcohol-ether	5	2.5 to 8.4	5.3	0	0	5
Benzoic acid	5	3.4 to 5.5	4.5	1	2	2
Tannic acid	5	3.6 to 6.4	5.1	4	1	0
Ba chloride	4	2.2 to 3.0	2.7	3	1	0
Na tungstate	5	4.6 to 7.5	5.6	4	0	1

Schering's thyrotropic hormone was added to 500 cc. normal urine in each method tried, the amount added being equivalent to 12 U for each chick and the injections of extract being given over 5 days.

Four volumes of acetone were added to the 24-hour output of urine which was then allowed to stand over night in the icebox. A packed precipitate resulted and the supernatant urine was decanted off. Fresh acetone was now added to the precipitate and the whole centrifuged. This was repeated twice, the acetone being discarded. The precipitate was then dried in vacuo and

made up to a suitable volume with water, the precipitate being made just alkaline to phenolphthalein; this was centrifuged and the supernatant fluid, after being neutralized was ready for injection.

The alcohol-ether and benzoic acid methods were similar to those described by Cope (20) and the tannic acid method was that described by Levin and Tyndale (29). As neither the precipitate nor supernatant liquid contained any demonstrable activity in either the barium chloride or sodium tungstate methods they need not be described.

An attempt was now made to recover a thyrotropic substance from the urine of 3 cases of myxedema and 2 cases of acromegaly. Either a half or the entire 24-hour output of urine was used for extraction with acetone and the injections were given daily for 5 days to chicks.

Table 4 summarizes the results obtained but only in case 2 was there any

TABLE 4. URINE FROM CLINICAL CASES

	B.M.R.	24-Hr. Output of Urine	Extraction Method	No. of Chicks	Thyroid				
					Weight Range	Mean Weight	Cytology		
		cc.			mg.	mg.	−	±	+
Case 1	−30	1,000	Acetone	2	3.5 to 5.0	4.3	1	1	0
Case 1	−30	900	Acetone	3	2.5 to 5.5	3.9	2	1	0
Case 2	−27	Fresh	urine used	5	2.6 to 5.2	3.8	5	0	0
Case 2	−27	1,950	Acetone	5	4.4 to 8.9	6.3	2	1	2
Case 2	−27	1,950	Tannic acid	3	2.6 to 5.5	4.2	1	2	0
Case 3	−25	650	Acetone	5	2.2 to 4.0	3.3	4	1	0
Case 4	+11	600	Acetone	4	3.6 to 5.5	4.5	3	1	0
Case 5	−30		Tannic acid	2	4.5 to 5.5	5.0	2	0	0

Twenty-four-hour output of urine was extracted by either acetone or tannic acid and the precipitate then injected into chicks.

indication of thyrotropic action. This case was a female, 65 years old, who had all the signs of a fully developed myxedema, had been given thyroid medication 3 years previously, but had taken no thyroid for 3 months prior to admission. Her B.M.R. was now −27%; blood iodine 5.8 mg. % and blood cholesterol 355 mg. %. Case 1, a woman 52 years old, had a somewhat similar history but had had only a few days' trial with thyroid which was discontinued when the patient complained of palpitation, sweating, and nausea. She had non-pitting edema of the face and extremities, loss of hair, and her B.M.R. was −30%; blood iodine 1.7 mg. % and blood cholesterol 226 mg. %.

Case 3 was a woman, 61 years old, who had had a total thyroidectomy performed 4 years previously to relieve cardiac decompensation. Her condition was one of hypothyroidism without myxedema. The B.M.R. has ranged from −40% to −25% since her operation and the blood cholesterol was 250 mg. %

Cases 4 and 5 were both acromegalics, one having a B.M.R. of +11% and the other a B.M.R. of −30%.

Table 5 shows the result of giving thyrotropic hormone together with

serum from schizophrenic patients; 2.0 cc. of serum were given daily for 3 days along with varying amounts of thyrotropic substance which was injected at a different site. Nine cases of schizophrenia were studied but in no instance was there any inhibition of thyrotropic activity, even when a minimal effective dose of the Schering thyrotropic substance was used.

TABLE 5. SERUM FROM SCHIZOPHRENICS

	B.M.R.	Dosage[1]	Number of Chicks	Thyroid				
				Weight Range	Mean Weight	Cytology		
				mg.	*mg.*	−	±	+
Normal Serum	—	4 U	5	2.5 to 6.0	4.6	2	3	0
Case 1		4 U	5	3.5 to 4.6	4.2	4	1	0
Case 2	−8	8 U	4	2.5 to 5.6	4.3	1	0	3
Case 3	−9	8 U	4	4.0 to 6.2	4.7	1	1	2
Case 4	−15	8 U	3	2.2 to 5.2	3.7	1	0	2
Case 5	−12	8 U	3	3.0 to 5.0	4.2	1	1	1
Case 2	−8	16 U	5	4.6 to 10.8	7.8	0	0	5
Case 3	−9	16 U	5	5.4 to 12.8	7.7	0	0	5
Case 4	−15	16 U	5	4.0 to 6.8	5.3	0	0	5
Case 5	−12	16 U	5	5.0 to 9.2	7.2	0	0	5
Case 6	−15	60 U	5	4.0 to 18.4	11.5	0	0	5
Case 7	−4	60 U	5	9.6 to 13.5	12.0	0	0	5
Case 8	−13	60 U	5	4.0 to 37.0	16.0	0	0	5
Case 9	−12	60 U	5	9.0 to 22.0	14.6	0	0	5

[1] Dosage in manufacturer's (Schering) units.

In all cases each chick received a total of 6.0 cc. of serum in 3 days, along with thyrotropic hormone injected at another site.

DISCUSSION

The satisfactory recovery by acetone precipitation of small amounts of thyrotropic substance which had been added to normal urine, makes it unnecessary to attempt more elaborate methods of extraction. It may be, however, that thyrotropic substance, if it is excreted in the urine, is in a form chemically quite distinct from that which was added in the Schering pituitary powder. With this in view, two rabbits were thyroidectomized and 4 months later the sera tested for thyrotropic activity. Unfortunately none had developed so that the efficiency of various extraction methods could not be tested by utilizing thyrotropic substance which was being excreted by the living animal. Cope (20), however, has described the development of thyrotropic activity in the serum of thyroidectomized rabbits.

If thyrotropic hormone is excreted in any considerable amount in certain pathological states occurring in man, and if when excreted its chemical properties are similar to the thyrotropic substance found in the anterior pituitary, then its detection ought to be reasonably certain even with the admittedly crude methods at our disposal. However, the presence of such a substance in human urine has never been established beyond doubt and it is difficult to believe that thyrotropic activity can be demonstrated by the injection of untreated urine into guinea pigs, rabbits, and hypophysectomized rats, as has been claimed by various workers. In the present series only one case of myxedema showed possible thyrotropic activity, but this could not be definitely

established because the patient was put on thyroid medication immediately after the urine sample was collected. It is interesting to note that Cope (20) and Spence (19) both failed to find thyrotropic activity in the serum or urine of cases of myxedema.

The failure to obtain any evidence of anti-thyrotropic activity in the serum of schizophrenic patients indicates that this has apparently no bearing on the thyroid resistance which they display. It would be interesting to know if they would be resistant to large doses of purified thyrotropic hormone, a situation which Scowen (30) has shown to be true in some cases of myxedema.

SUMMARY

Several methods for the extraction of pituitary thyrotropic hormone added to normal urine are described; acetone and alcohol-ether methods gave a very satisfactory recovery, as judged by the response of the chick thyroid.

Urines from 2 cases of myxedema, 1 case of post-operative hypothyroidism, and 2 acromegalics were tested for thyrotropic activity; 1 case of myxedema gave results suggesting possible thyrotropic activity.

Sera from 9 schizophrenic patients did not show any anti-thyrotropic activity when injected into chicks along with known amounts of thyrotropic substance.

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INFLUENCE OF HYPERTHYROIDISM ON VITAMIN C CONTENT OF VARIOUS ENDOCRINES AND TISSUES[1]

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DURING the past two and a half years this laboratory has been engaged in investigations on oxidation-reduction efficiency in avitaminoses which included studies on the ascorbic acid[2] concentration in blood, tissues, and endocrine glands. Although it is generally recognized that the rat synthesizes vitamin C, we are finding considerable reduction of this vitamin in certain tissues in vitamin A and B_1 deficiencies (1), thus establishing a vitamin interrelationship. In continuing our hyperthyroid studies, it occurred to us that toxic doses of thyroxine may so damage certain tissues or endocrines as to produce a marked reduction in the vitamin C content. This was found to be true in the case of the kidney, liver, heart, adrenals, and thymus and to a lesser extent and less regularly in the thyroids and pituitary, when all the work completed is taken into consideration. That there is a greater requirement of vitamin B_1 in hyperthyroidism has been reported in previous publications (2). The question then presented itself: do hyperthyroid rats require greater amounts of other vitamins? In this communication, data are submitted which show an interrelationship between vitamins B_1 and C in hyperthyroidism and in vitamin B_1 deficiency.

EXPERIMENTAL

The majority of the experiments were conducted on our vitamin B_1 deficient diet 3148-xxx[3] as the basal ration with and without thryoxine and with various vitamin additions. Every animal received 4 drops of Mead's cod liver oil daily. Five sets of experiments were conducted with albino rats in groups of 3, 13, in sets of 4, and 6 in groups of 6. These animals when started on the experiments ranged in age from 24 to 44 days and in weight from 63 to 98 gm. Two brands of thyroxine were employed, natural supplied by E. R. Squibb and Sons[4] and the racemic synthetic compound furnished by Hoffman-La Roche Inc. The natural (*l*-thyroxine) was given in daily doses of 0.1 to 0.2 mg. The synthetic product was administered in larger daily doses, 0.25 to 0.5 mg., in view of Harington's statement (3) that *l*-thyroxine is 1.5 times as potent as the racemic compound.

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[2] The terms 'ascorbic acid' and 'vitamin C' are used interchangably in this paper.

[3] Composition of ration 3148-xxx: casein (purified) 16; salts #185, 4; Fleischman's yeast, autoclaved for 5 hr. at 15 lbs. pressure, 7; beef autoclaved for 6 hr. at 20 lbs. pressure, 8; filtered butter fat, 9; dextrinized starch, 56.

[4] We appreciate the cooperation of E. R. Squibb and Sons who kindly supplied the *l*-thyroxine (natural) used in this research.

The influence of hyperthyroidism on the ascorbic acid content of various tissues and endocrines was also studied on adult rats on stock diet No. 2,[5] using pairs of adult animals, with additions of chiefly 1 mg. daily of the racemic thyroxine. The adult rats were 87 to 122 days of age when started on the thyroxine experiments and weighed 168 to 240 gm.

In order to determine if it is possible to increase the vitamin C content of tissues and endocrines of the rat on a highly satisfactory stock diet,[6] 16 rats were allowed daily doses of ascorbic acid of 4 to 16 mg. daily for periods varying from 36 to 66 days, without any notable change in the concentration of vitamin C either in the adrenals, thymus, thyroids, pituitary, pancreas, lung, liver, kidney, or heart. Neither did starvation for as long as 10 to 11 days show any influence on reduction of ascorbic acid in the tissues and endocrines mentioned above. This was determined in 5 sets of experiments on the stock diet ad libitum. Space does not permit presenting all of our data in detail. Therefore, only representative results are submitted in tables 1, 2, and 3.

Preparation of Tissue Extracts and Methods of Ascorbic Acid Determinations

For an extractive of ascorbic acid we used an 8% solution of trichloracetic acid containing 4% metaphosphoric acid. Such a mixture was found by Musulin and King (4) to be an efficient solvent and at the same time insures stability of ascorbic acid. Since there was always sufficient liver and kidney, 0.5 gm. samples of these organs were taken, ground in a small mortar with small volumes of the extractive and a little sand, and diluted to 10 cc. in graduated 15 cc. centrifuge tubes. Of the heart, varying quantities were available. The dilution used was approximately 1 cc. for every 100 mg. of tissue. The adrenals were diluted 1:100 and the thyroids and pituitary 1:200. For the adrenals porcelain crucibles were used as mortars. As mortars for the thyroids and pituitary we used test tubes, having 1 cm. bore cut down to 3 cm. in length, and bent at the opening in the flame, so as to provide lips for transferring the endocrine extracts into the centrifuge tubes. After shaking for 3 minutes, the supernatant liquids were decanted and ascorbic acid determined with the 2-6 dichlorophenolindolphenol dye. The macro method of titration was employed for the organs and the micro method of Farmer and Abt (5) developed for blood was adopted for the endocrines.

Influence of Hyperthyroidism on the Vitamin C or Ascorbic Acid Content of Various Endocrines and Tissues

The results submitted in tables 1 and 2 were secured with rats on a diet deficient only in vitamin B_1. This diet was supplemented with 6 drops of cod-liver oil daily to each animal. The positive control received 20 γ of vitamin B_1 without any thyroxine. Since other animals in the various set ups received varying amounts of vitamin B_1 and ascorbic acid additions in the presence of thyroxine, it is possible to evaluate the contributions made by these vitamins in antagonizing the toxic influence of the thyroid hormone in reducing the vitamin C content of the various endocrines and tissues.

Group 3929 (table 1). The *F* animal has a double disadvantage. It was on a vitamin B_1 deficient diet and received a 0.5 mg. of synthetic thyroxine daily. It is evident that a reduction in the concentration of ascorbic acid has taken place in all the endo-

[5] Composition of stock diet 2: whole wheat, 66.7; whole milk powder, 32.0; sodium chloride, 1.3.

[6] Our cereal stock diet 1 was used of the following composition: whole wheat, 27; rolled oats, 26; yellow corn, 25; flaxseed meal, 15; commercial casein, 5; cod-liver oil, 1; NaCl, 1; $CaCO_3$, 0.5, liberal supply of whole milk daily.

TABLE 1. INFLUENCE OF HYPERTHYROIDISM ON VITAMIN C CONTENT OF VARIOUS ENDOCRINES AND TISSUES

Animal marking and sex	Days on thyroxine	Vitamin supplement	Change in weight % of BW[5]	Adrenals		Thymus		Thyroids		Pituitary		Lung		Kidney		Liver		Heart	
3929[1]																			
F[2]	18	—	−45.0	2.62[3]	−35.8[4]	.21	−32.2	.23	−30.3	.72	−34.6	—	—	.08	−33.3	.14	−26.3	.05	−54.5
L·F	18	20 γ B_1	+29.4	2.92	−28.4	.28	− 9.7	.28	−15.1	.85	−22.8	—	—	.12	± 0.0	.22	+15.7	.10	− 9.1
R·F	18	20 γ B_1 +2 mg. VC	+23.3	3.70	− 9.3	.21	−32.2	.23	−30.3	1.00	− 9.1	—	—	.08	−33.3	.17	−10.5	.09	−18.2
D·F	—	20 γ[7] B_1	+71.1	4.08	—	.31	—	.33	—	1.10	—	—	—	.12	—	.19	—	.11	—
3941[1]																			
L·M	7	—	−38.1	2.62	−14.9	.15	−65.1	—	—	.64	−53.6	.15	−25.0	.04	−63.6	.12	−45.4	.04	−55.5
D·M	7	6 mg. VC	−16.9	3.00	− 2.3	.20	−53.5	—	—	1.10	−20.3	.20	± 0.0	.08	−27.2	.19	−13.5	.09	± 0.0
M	7	6 mg. VC +100 γ B_1	+64.4	4.20	+36.3	.33	−23.2	—	—	1.40	+ 1.5	.36	+80.0	.13	+18.2	.29	+31.8	.11	+22.2
R·M	—	20 γ B_1	+76.9	3.08	—	.43	—	—	—	1.38	—	.20	—	.11	—	.22	—	.09	—
3942[6]																			
R·F	6	—	−43.4	2.33	−22.3	.15	−62.5	.21	−25.0	.71	−11.2	.13	−38.0	.06	−40.0	.11	−26.6	.04	−33.2
D·F	6	6 mg. VC	−27.6	3.70	+23.3	.15	−62.5	.22	−21.4	.87	− 8.7	.21	± 0.0	.06	−40.0	.21	+40.0	.07	+16.6
F	6	6 mg. VC +100 γ B_1	+64.3	4.16	+38.6	.24	−40.0	.31	+10.7	.98	+22.5	.27	+28.6	.12	+20.0	.22	+46.6	.10	+66.6
L·F	—	20 γ B_1	+81.5	3.00	—	.40	—	.28	—	.80	—	.21	—	.10	—	.15	—	.06	—

Basal diet was vitamin B_1 deficient. 6 drops cod-liver oil daily for each animal. VC = vitamin C or ascorbic acid.

[1] 0.5 mg. synthetic thyroxin daily.
[2] *M* = male; *F* = female.
[3] Expressed as mg. of ascorbic acid per gm. of fresh tissue.
[4] Percentage change.
[5] BW = body weight.
[6] 0.25 mg. synthetic thyroxine daily.
[7] γ = gamma or 1,000th of a milligram.

TABLE 2. INFLUENCE OF HYPERTHYROIDISM ON CONCENTRATION OF VITAMIN C IN VARIOUS TISSUES AND ENDOCRINES

Exp. No.	Animal marking and sex	Thyroxine (mg.)	Days on thyroxine	Daily vitamin supplement	Change in weight % of BW[1]	Thymus		Adrenal		Kidney		Liver		Heart	
						% change		% change		% change		% change		% change	
4040	*M*[2]	—	—	—	−21.3	.09[2]	−35.7	3.06	−18.2	.10	−41.1	.19	−29.6	.08	−20.0
	L-M	0.1 n[4]	15	—	−32.3	.05	−64.3	2.72	−27.3	.07	−58.6	.17	−37.0	.07	−30.0
	D-M	0.1 n	15	6 mg. VC	−21.4	.07	−50.0	3.40	− 9.1	.11	−35.3	.26	− 3.7	.10	± 0.0
	DL-M	0.1 n	15	6 mg. VC +20 γ B$_1$	+65.6	.20	+42.8	3.51	− 6.2	.14	−17.6	.29	+ 7.4	.12	+20.0
	DR-M	0.1 n	15	6 mg. VC +100 γ B$_1$	+64.5	.24	+71.6	4.25	+13.6	.14	−17.6	.29	+ 7.4	.12	+20.0
	R-M	—	—	20 γ[5] B$_1$	+48.6	.14	—	3.74	—	.17	—	.27	—	.10	—
4042	*F*	—	—	—	−15.7	.06	−78.6	2.60	−27.7	.10	−23.0	.19	−13.6	.08	± 0.0
	L-F	0.2 n	14	—	−34.7	.05	−82.1	2.34	−35.0	.07	−46.1	.17	−22.7	.05	−37.5
	R-F	0.2 n	14	6 mg. VC	−11.2	.07	−75.0	2.47	−31.3	.10	−23.0	.19	−13.6	.10	+25.0
	D-F	0.2 n	14	6 mg. VC +20 γ B$_1$	+22.9	.18	−35.5	3.38	− 6.1	.10	−23.0	.25	+13.6	.10	+25.0
	DL-F	0.2 n	14	6 mg. VC +100 γ B$_1$	+24.2	.24	−14.3	3.60	± 0.0	.11	−15.4	.21	− 4.5	.09	+12.5
	DR-F	—	—	20 γ B$_1$	+30.2	.28	—	3.60	—	.13	—	.22	—	.08	—
4051	*L-M*	—	—	—	−31.0	.22	−45.0	3.20	−20.0	.09	−50.0	.16	−42.5	.06	−33.3
	DL-M	0.2 n	9	—	−35.0	.13	−67.5	2.66	−33.5	.06	−66.6	.11	−60.7	.05	−44.4
	D-M	0.2 n	9	20 γ B$_1$	+36.4	.28	−30.0	3.84	− 4.0	.13	−27.7	.17	−39.2	.12	+33.3
	R-M	0.2 n	9	6 mg. VC +20 γ B$_1$	+30.0	.28	−30.0	3.84	−4 .0	.11	−38.8	.22	−21.4	.10	+11.1
	DR-M	0.2 n	9	6 mg. VC +100 γ B$_1$	+43.6	.29	−27.5	4.00	± 0.0	.13	−27.7	.26	− 7.1	.11	+22.2
	M	—	—	20 γ B$_1$	+46.5	.40	—	4.00	—	.18	—	.28	—	.09	—
4057	*M*	—	—	—	−13.6	.27	−43.7	3.04	− 9.5	.11	−42.1	.19	−38.7	.09	−30.7
	L-M	0.2 n	15	—	+ 7.6	.22	−52.1	2.88	−14.3	.07	−63.2	.16	−48.4	.08	−38.4
	D-M	0.2 n	15	20 γ B$_1$	+80.0	.33	−31.2	3.84	+14.3	.14	−26.3	.29	− 6.5	.09	−30.7
	DL-M	0.2 n	15	6 mg. VC +20 γ B$_1$	+80.0	.40	−16.7	3.68	+ 9.5	.14	−26.3	.29	− 6.5	.17	+30.7
	DR-M	0.2 n	15	6 mg. VC +100 γ B$_1$	+67.8	.37	−22.9	3.84	+14.3	.14	−26.3	.30	− 3.2	.12	− 7.7
	R-M	—	—	20 γ B$_1$	+82.4	.48	—	3.36	—	.19	—	.31	—	.13	—

Basal diet was vitamin B$_1$ deficient. 6 drops of cod-liver oil daily to each animal. VC=vitamin C or ascorbic acid.

[1] BW=body weight.
[2] Mg. of ascorbic acid per gm. of fresh tissue.
[3] *M*=male; *F*=female.
[4] n=natural thyroxine.
[5] γ=gamma.

TABLE 3. INFLUENCE OF HYPERTHYROIDISM ON CONCENTRATION OF VITAMIN C IN THYMUS AND VARIOUS TISSUES
(on Stock Diet No. 2)

Exp. No.	Animal marking and sex	Thyroxine (mg.)	Days on thyroxine	Change in weight % of BW[6]	Thymus		Kidney		Liver		Heart	
					M.B.[5]	D.B.[5]	M.B.	D.B.	M.B.	D.B.	M.B.	D.B.
4049	*P-R M*[1]	1 mg. n[2]	21									
		0.5 mg. n	7	−3.4	.29[3] −12.1[7]	1.07[4] −33.5[7]	.10 −28.5	.40 −29.8	.24 − 7.7	.84 ± 0.0	.11 +10.0	.30 −33.3
	C-D M	—	—	+36.7	.33	1.61	.14	.57	.26	.84	.10	.45
4043	*P-R M*	1 mg. n	27									
		0.5 mg. n	5	−11.2	.21 −46.1	0.66 −55.1	.05 −58.3	.25 −46.9	.11 −54.1	.39 −49.2	.06 −60.0	.26 −58.1
	C-D M	—	—	+31.2	.39	1.47	.12	.47	.24	.77	.15	.62
4033	*P-M*	1 mg. n	38									
		0.5 mg. n	4									
		2 mg. s[2]	1	+ 4.7	.43 −23.2	1.61 −30.6	.11 −38.9	.50 −30.5	.25 −16.6	.86 −11.4	.10 +11.1	.50 +19.0
	C-M	—	—	+51.4	.56	2.32	.18	.72	.30	.97	.09	.42
4058	*P-F*	1 mg. n	19									
		0.5 mg. n	4									
		2 mg. s	1	−8.9	.17 −56.4	0.65 −60.8	.08 −50.0	.35 −47.7	.16 −36.0	.62 −27.9	.07 −22.2	.29 −30.9
	C-L M	—	—	+8.9	.39	1.66	.16	.67	.25	.86	.09	.42
		Average			−34.4	−44.9	−43.9	−38.7	−28.6	−21.9	−15.0	−30.0

[1] *P*=pathological; C=control; *M*=male; *F*=female.
[2] n=natural thyroxine; s=synthetic thyroxine.
[3] Mg. of ascorbic acid per gm. fresh tissue.
[4] Mg. of ascorbic acid per gm. dry tissue.
[5] M.B.=moist basis; D.B.=dry basis.
[6] BW=body weight.
[7] Per cent change.

crines and tissues. The introduction of 20 γ of vitamin B_1 to *L-F* checked the reduction of ascorbic acid content in all endocrines and tissues, most notably in the thymus, and the heart. The influence of thyroxine can be determined by examining the difference in results secured between those on animal, *L-F* and those on rat *D-F*, the latter having had the same 20 γ of vitamin B_1 addition but without thyroxine administration. Losses due to thyroxine are apparent in all instances with the exception of the liver where there is a slight gain in vitamin C content but this is the exception rather than the experience with most of the animals. The results on the addition of 2 mg. of ascorbic acid are irregular and cannot be interpreted.

Group 3941 (Table 1). Here again, as in experiment 3929, and which is true of all the rest of the experiments reported in this paper, toxic doses of thyroxine, synthetic or natural, administered to animals on a vitamin B_1 deficient diet, produce tremendous reductions in the ascorbic acid content of all the endocrines, kidney, liver, lung, and heart. The special points of interest in this experiment, however, are: the addition of 6 mg. vitamin C (Merck's cebione) even in the absence of vitamin B_1, markedly reduced the great losses of ascorbic acid in the tissues and the pituitary but to a lesser extent in the adrenals and thymus. The addition of 100 γ vitamin B_1 (as a supplement to the 6 mg. vitamin C) to animal *M*, which is 5 times the dose given to the positive control (*R-M*), increased the ascorbic acid content above the control in all instances with the exception of the pituitary where the slight gain is insignificant. The vitamin C addition to the daily diet also reduced the loss of body weight from 38.1 to 16.9%.

Group 3942 (table 1). Whereas the daily dose of synthetic thyroxine in groups 3929 and 3941 was 0.5 mg., in the case of group 3942 it was 0.25 mg. In this experiment the addition of a daily dose of 6 mg. vitamin C had a marked influence on the ascorbic acid content of the adrenals, lung, liver and heart. In the case of the latter two tissues the ascorbic acid was increased above that of the control (*L-F*) even in the absence of vitamin B_1. The further addition of 100 γ of vitamin B_1 resulted in further increases of vitamin C in all instances. Since vitamin B_1 influences the vitamin C content of endocrines and tissues, we thus have a definite picture of vitamin interrelationship. It is also clear from this experiment that the vitamin C addition reduced the loss of weight by 25%.

Groups 4040, 4042, 4051, and *4057* were studied in sets of 6 which were litter mates of the same sex. Since in these sets one animal received a vitamin B_1 deficient diet without thyroxine, it was possible to note the differences in ascorbic acid content of the endocrines and tissues as influenced by vitamin B_1 deficiency without the complication of thyrotoxicosis.

Groups 4040 and 4042 (table 2). The influence of both vitamin B_1 deficiency and of thyroxine on the reduction in the ascorbic acid content of endocrines and tissues is quite apparent. The B_1 deficiency, however, had a greater influence than thyroxine in vitamin C losses. It is also apparent that both vitamins B_1 and C contributed to the checking of reduction of ascorbic acid in all instances. The protective action of the increasing dosage of vitamin B_1 is also evident in all cases with the exception of the heart. The increased values in the heart when the animals in these groups received only 20 γ of vitamin B_1, in the presence of thyroxine, are difficult to explain, unless they were influenced by changes in water metabolism. While both vitamins C and B_1 produced their individual effects on body weight, the increasing amount of vitamin B_1 exerted no such influence. It will be noted that neither on the 0.2 mg. daily dose nor the 0.1 mg. dose of thyroxine was there entire protection produced by additions of vitamins C and B_1 in the case of the kidney. This is also true of groups 4051 and 4057.

Groups 4051 and 4057 (table 2). It will be noted from these experiments that addition of 20 γ of vitamin B_1 alone produces a tremendous influence on the vitamin C metabolism of the thyrotoxic rat. This is evidenced in all instances. When the experimental period on 0.2 mg. natural thyroxine was increased from 9 to 15 days the protective action of increasing amounts of vitamin B_1 is less apparent (*DR-M*, *4057* and *DR-M*, *4051*). The protective action of vitamin C against ascorbic acid losses, when it fortifies vitamin B_1, is also brought out in these experiments, but the greater effect is produced by vitamin B_1 than by vitamin C additions. In these two sets of experiments vitamin C did not exert any influence on increases in body weight. A daily dose of 100 γ of vitamin B_1 and 6 mg. ascorbic acid protected practically all losses of vitamin C in the adrenals, liver and heart but not in the kidney.

Ascorbic acid determinations were made on the dry as well as the wet basis in all the groups shown in table 2 and on two additional sets not submitted in this table for lack of space. The results on the dry basis are omitted because, as a whole, the data were consistent in all cases with the exception of the adrenals where changes in water metabolism rendered such comparisons impossible.

From table 3 it is clear that marked reductions in ascorbic acid content in the thymus, kidney, liver and heart in hyperthyroidism are not due to changes in water metabolism. Groups *4049*, *4043*, *4033*, and *4058* were on a stock diet, which when supplemented with lettuce once a week, supports growth, reproduction, and lactation in the albino rat. The animals were studied in pairs and thyroxine was the only factor responsible for ascorbic acid losses in the pathological animals. It will be noted that the losses are evident on the dry as well as the wet basis, and more on the dry basis in the case of the thymus and heart.

It is apparent from the experimental evidence submitted that in hyperthyroidism there is a depletion of vitamin stores of the body and that not only is more vitamin B_1 required to prevent the rapid catabolism but additional beneficial results are secured by vitamin C administrations. The fact that the vitamin C content of the endocrines and tissues of the rat (which does not require ascorbic acid on a normal diet) suffers tremendous losses of this vitamin following toxic doses of thyroxine, prompts us to suggest to clinicians to administer ascorbic acid in larger doses as a supplement to vitamin B_1 (thiamine) to human cases of toxic goiter, particularly the non-operative types. Whether there are greater requirements of other vitamins in hyperthyroidism is now being investigated in our laboratory.

SUMMARY

Two factors were found responsible for tremendous reductions of ascorbic acid in endocrines and tissues: *a*), vitamin B_1 deficiency and *b*), thyrotoxicosis. Administrations of vitamins B_1 and C counteracted to a large extent such ascorbic acid losses in the body of the rat. The fact that one vitamin (B_1) deficiency can influence marked reduction of another vitamin (C) establishes definitely a vitamin interrelationship.

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THE BLOOD CHOLESTEROL IN THYROIDECTOMIZED RATS AS RELATED TO THE EFFECTIVENESS OF GONADOTROPIC HORMONES[1]

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IT HAS BEEN shown that experimental hyperthyroidism in the rat exerts an inhibiting effect on the action of the gonad stimulating hormones (1, 2). Moreover, thyroidectomy is followed by an increase in the effectiveness of the follicle stimulating hormone but not in that of pregnancy urine or pregnant-mare serum (1, 3, 4). In view of the knowledge that these gonadotropic substances are different in their action on the ovary, other factors than decreased metabolism were sought to explain the difference in the effect of thyroidectomy.

Our attention was directed to the almost universal observation that hypothyroidism in many animals and in man is accompanied by an increase in the cholesterol of the blood (5, 6). Since cholesterol is closely related chemically to the sex hormones and hence ovarian function, it seemed practical to investigate blood cholesterol changes in the rat in relation to thyroidectomy and gonadotropic hormone response. Although it has been shown that hyperthyroidism lowers the blood cholesterol in the rat (7), no references were found indicating that hypercholesterolemia follows thyroidectomy in this species. This report will deal with: *a*), the effect of feeding cholesterol on the response of follicle stimulating hormone and *b*), blood cholesterol changes in rats after thyroidectomy.

METHODS

Total blood cholesterol determinations were made by extracting dried samples of blood with chloroform in an apparatus similar to that described by Abrahamson (8). For each determination 0.5 cc. of blood was taken from the heart and duplicate samples of 0.2 cc. were spread on filter paper to dry. After extraction with chloroform the characteristic color was developed by means of the Liebermann-Burchard reaction. Color comparisons were made in a standard colorimeter. This micro-method, using only 0.2 cc. of whole blood, gave results which were comparable with those obtained by the digitonin precipitation method in which larger quantities of blood were used. For example, a blood sample yielded 93 mg. of cholesterol per 100 cc. of blood by the latter method as compared to 97 mg. per 100 cc. by the micro-method. Since the amount of blood obtainable from the small animals was limited and since

[1] This work was supported by a grant from the Penrose Fund of the American Philosophical Society.

our interest was not in the partitioning of free cholesterol and the esters but in the changes in the total cholesterol of the blood, the digitonin method was not used.

In all the experiments litter-mate animals were used. In the first experiment, one group of immature female rats received cholesterol at the rate of 200 mg. per rat per day for 8 days in addition to their regular diet of Purina dog chow. The controls received only the regular diet. On the 9th day equal amounts of follicle stimulating hormone (tannate preparation from menopause urine) were injected into each group of rats and the cholesterol feeding continued. At the end of 4 more days the animals were killed and their ovaries weighed. Blood samples for cholesterol determinations were taken from both groups of rats, before and at the end of the experiment.

In the second experiment, young adult females, immature females and one litter of males were grouped into control and thyroidectomized series. Blood samples were taken from the heart before removal of the thyroid and at different periods thereafter. Examination of the thyroid site at the end of the experiment in all cases showed no trace of thyroid tissue.

RESULTS

From the results in table 1, it is seen that feeding of cholesterol in addition to the regular diet did not alter the concentration of cholesterol in the blood or the effect of the follicle stimulating hormone (FSH). The amount

TABLE 1. CHOLESTEROL FED RATS AND OVARIAN RESPONSE TO FSH[1]

Exp. No.	Age of rats *days*	Av. Blood Cholesterol mg./100 cc.				Av. ovarian Wt. (*mg.*)	
		At Beginning of Experiment		At Autopsy		Control	Exper.
		Control	Exper.	Control	Exper.		
R 22	25	(4)[2] 104	(3) 107	91	94	29.8	22.8
R 26	22	(5) 93	(5) 90	105	107	46.6	46.9

[1] FSH, 40 mg. tannic acid ppt. of menopause urine equiv. per 100 cc.
[2] Number of rats.

of cholesterol fed probably was not sufficient to raise the blood cholesterol level. Further feeding experiments were not attempted in view of the other results obtained.

Table 2 shows the blood cholesterol changes in male and female rats occurring at varying periods after thyroidectomy. There seems to be no hypercholesterolemia in the rat after removal of the thyroid. In every case where there appears to be a rise in blood cholesterol, it is noted that a similar rise occurs in the blood of the litter-mate controls. The degree of variation in both groups is about the same with overlapping of the extreme limits. The average blood cholesterol for all the control rats is 105 mg. per 100 cc. while that of the thyroidectomized group is 98 mg. per 100 cc. In both the operated and control series, it is noted that as the rats grew older, the level of cholesterol tended to fall.

DISCUSSION

The failure of cholesterol feeding to raise the blood cholesterol level may be attributed to an insufficient amount of the material being fed for an

inadequate period of time. In other feeding experiments reported, a high blood cholesterol was usually obtained only after prolonged periods of feeding. Since our object was to determine whether high blood cholesterol was related to the responsiveness of the immature ovary to FSH the period of feeding had to be limited. Prolonged feeding of cholesterol to rats has resulted in a lengthening of the estrus cycle by 1 to 2 days, a slight interference with intra-uterine growth but no effect on male reproductive activity

TABLE 2. BLOOD CHOLESTEROL VALUES FOLLOWING THYROIDECTOMY

Exp. no.	No. of rats		Age rats days	Total Blood Cholesterol (mg./100 cc.)						Days after operation
				Control			Exper.			
	Cont.	Exp.		Min.	Max.	Av.	Min.	Max.	Av.	
R 7, Male	3	3	45	83	90	87	68	88	75	8
	3	3	67	100	107	104	94	102	97	30
R 21, Fem.	2	3	25	104	123	114	90	111	100	—
	2	3	33	97	106	102	84	102	94	6
	2	2	54	115	121	118	119	140	130	27
	2	2	83	115	115	115	113	131	122	56
	2	2	89	111	117	114	81	120	100	62
	2	2	114	91	91	91	80	84	82	87
R 30, Fem.	3	4	31	96	98	97	101	114	108	—
	3	3	44	117	132	122	120	125	123	12
	3	3	81	94	104	101	98	100	99	37
	3	3	104	79	99	89	81	88	84	60
R 33, Fem.	3	4	105	112	131	123	102	110	106	—
	3	4	115	90	130	109	101	112	106	8
	3	4	156	88	98	94	80	93	86	49
R 35, Fem.	3	3	34	125	128	126	92	117	103	—
	3	3	84	89	102	95	86	102	92	50

(9). Further work in this direction was abandoned when it was observed that thyroidectomy alone failed to raise the cholesterol level of the blood.

The blood cholesterol of the rats in these experiments did not show the usual marked increase that has been reported to accompany hypothyroidism in other mammals, that is man, rabbit, guinea pig, cat and dog. For example, the hypercholesterolemia reported to occur in operated dogs is consistently higher than the controls (5). One operated rat in litter *R 21* had a high blood cholesterol (140 mg. per 100 cc.) which appears significant yet one control rat in litter *R* 30 showed a level almost as high (132 mg. per 100 cc.). The average of all the normal rats was 105 mg. per 100 cc. whereas that of the thyroidectomized rats was 98 mg. per 100 cc. of blood.

It was observed that whenever a change occurred in the blood cholesterol of the operated rats after periods of hypothyroidism, almost invariably a similar change of about the same magnitude occurred in the controls. Since blood samples were taken from operated and control animals at each interval, as well as before thyroidectomy, the changes in cholesterol level due to the operation appear insignificant. Such environmental conditions which might

alter the results were thus controlled. If this procedure had not been followed a different interpretation would have resulted.

In general, it was seen that the cholesterol level tends to be higher in younger animals than in older animals. This was true for the operated as well as the control animals. Comparison of the average initial blood cholesterol in both groups, with that of the last determination in each group (excepting *R* 7) shows an average drop of 23 mg. per 100 cc. in the control group and 18 mg. per 100 cc. in the operated group. Shope (10) has reported that a rapid increase in blood cholesterol occurs in early life in the cow, rabbit and guinea pig but with advancing age, there is a definite decline.

The suggestion that cholesterol may be acting as mediator of the thyroid-ovarian relationship has been made by Parhon et al. (11). They found that the cholesterol content of the guinea pig ovary was doubled after thyroidectomy but that thyroid feeding for 20 days had little or no effect. It is possible that similar studies in the rat may add to the understanding of this endocrine relationship. This is suggested from the work of Anderson and Sperry (12) in which they point out that there is no evidence for a direct relationship between blood serum and adrenal cholesterol in the rat although a significant variation occurs in the adrenal cholesterol during phases of the reproductive cycle. In spite of the unique results obtained in the blood cholesterol study of hypothyroid rats, the possibility that cholesterol may play a part in the hypophyseal-thyroid-ovarian relationship has not been eliminated.

SUMMARY

Feeding of cholesterol at the rate of 200 mg. per day for 12 days did not alter blood cholesterol or effect the responsiveness of the rat ovary to injected follicle stimulating hormone. There appeared to be no significant change in total blood cholesterol in the rat following thyroidectomy. Such changes that occurred were found in both experimental and control animals. The responsiveness of the ovary to follicle stimulating hormone increases after thyroidectomy in the rat but this does not appear to be correlated with the level of total cholesterol in the blood.

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THE CALORIGENIC EFFICIENCY OF THYROID MATERIAL IN RELATION TO THYROXINE AND TO IODINE CONTENT

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Although thyroxine possesses most, if not all, of the physiological effects on organisms, that are obtained with whole thyroid gland, the general impression prevails that crude thyroid material, when administered orally, exerts an action that is not quantitatively in agreement with its thyroxine content and possibly even qualitatively of a more or less different character.

Thyroxine content of glandular material of standard iodine content (U.S.P. 0.2% iodine) is subject to wide variations, and consequently no physiological uniformity can be expected unless one rejects the theory that the thyroxine is of any outstanding significance in thyroid action. In fact, Salter, Lerman and Means (1) claimed that clinically the total iodine rather than the thyroxine content of thyroid substance was the better index of its ability to relieve myxedema. However, since Thompson, et al. (2) could show that alkaline hydrolysis decreases the activity of dried thyroid considerably, while the total iodine content remained unchanged and the possible destruction of thyroxine was relatively small, the probability that the chemical structure of a complex molecule is the deciding factor cannot be rejected.

Palmer and Leland (3) and Foster, Palmer and Leland (4) claim strict identity of calorigenic action of thyroid material and its thyroxine content if the latter is calculated as the higher active l-thyroxine and determined by the method of Leland and Foster (5). However, this latter method gives values admittedly about 15% too low. The values, as they used them, should consequently have been increased by about 18%, which would have made the agreement less conspicuous, although the variations might very well have remained within the limits of error. The fact that one of their commercial thyroid preparations showed a surprisingly high activity, remains unexplained.

It cannot be denied that the chemical methods of assaying thyroid material are not only inadequate, but that the relationship between chemical composition and physiologic activity is still obscure, or at least debatable. The bioassay, therefore, must be a necessary supplement in the evaluation of thyroid preparations. It is not within the scope of this paper to enumerate and discuss the various methods proposed for this purpose. The limits of error are generally too wide to justify their practical application.

In a preliminary paper (6) we published a report on experiments carried out on normal and thyroidectomized rats in which the increase in basal metabolism before and after feeding of thyroxine and thyroid globulin was compared. In the following we give a complete account of this work, which includes also the testing of various other thyroid preparations leading to a comparison of their relative calorigenic potency.

METHOD

A closed circuit apparatus, as described by Benedict (7) was used for the determination of metabolism. The rats were of a highly inbred Wistar strain, kept on a diet of 3 parts of whole wheat and 1 part of dried whole milk, supplemented weekly by lean meat and lettuce. The temperature of the animal room was maintained above 20°. Moderate variations of temperature above that limit did not seem to have a noticeable influence on the rats, but extreme summer heat—approaching 30°—made it necessary to interrupt the tests, since the animals became restless and unreliable. For the test, the animals were starved for 20 hours and then placed in a 1-quart Mason jar provided with air inlet and outlet, connected with the circuit, and thermometer. The jar was submerged in a water bath of a constant temperature of 30°. A strong electric light bulb was placed over the chamber, since the rats are more inclined to be restful in a bright light than in the dark. After a preliminary period of at least 30 minutes, in which the animal became calm and attained respiratory equilibrium, the time necessary to consume a standard volume of oxygen was measured, (about 240 mg. O_2) slightly varying with room temperature and barometric pressure. The corrected weight of oxygen consumed was calculated for mg. oxygen consumed per kg. body weight per hour. Tentative calculations on the basis of body surface instead of weight gave figures which were not more consistent and consequently were not used.

Tests were counted only if the animal was at rest but not asleep, and when uniformity was observed between several consecutive runs. The test was repeated several days afterwards and had to agree with the previous results before a figure for basal metabolism was considered as acceptable. Some rats had during the first test a higher metabolism, but gave lower and more constant values later on after being accustomed to the procedure. Only male rats were used. The normal rats were of an age between 80 and 100 days when started. It was found that the metabolism of those between 70 and 80 days old is usually high and variable, probably due to the onset of maturity. Afterwards rather uniform values could be obtained. Thyroidectomy was performed when the animals were 65 to 80 days old. With a diet rich in calcium in the form of milk, the incidence of tetany could generally be kept low so as not to interfere with the tests. Contrary to the statements of Gaddum (8), it was found that 2 weeks after the operation the metabolism had not yet reached the lowest level. The animals were not used until 4 weeks had elapsed. At that time the consumption of O_2 in mg. per kg. weight per hour varied between 880 and 1200, as compared with values of 1420 and 1670 in normal rats. A certain percentage of animals was found to be too unstable to give uniform results, be it because of nervousness and excitability, tendency to tetanic attacks, or for some process of disease. Such rats were discarded and never used for the experiments.

Medication with thyroid material was given by stomach tube for 3 consecutive days, the daily dose being calculated in γ per each 10 gm. of body weight. The metabolism was determined again on the 5th day and the percentage increase in metabolism calculated.

In view of the fact that a rather long time is required until an animal is depleted of thyroid hormone, and considering that a subthreshold remainder

TABLE 1

Dose γ per 10 gm. per dose	Given on days	Metabolism mg. O_2 per hr. per kg.				
		before treatment	on day			
31	1st	1600	4th	1600	7th	1600
31	1st	1580	3rd	1720		
22 injected	1st–2nd	1400	4th	1680	7th	1640
21 injected	1st	1680	3rd	1920	6th	1760
28	1st	1480	4th	1830	7th	1580
31	1–2–3	1600	5th	2260	8th	1750
15	1–2–3	1600	5th	1900	8th	1500

Although these experiments were too few to give a picture of the metabolic reaction to medication, it seems to be apparent that on the 6th day after medication the metabolism returned to, or approached, the pre-treatment level. A 3-day treatment with test on the 5th day was chosen as standard procedure. The following table 2 includes the last 2 rats of table 1.

of no noticeable influence on metabolism, but still able to complicate the effect of subsequent treatment, might stay for a non-determinable time, it was preferable to use an animal only once. Later, we found that this precaution was not absolutely necessary.

Materials used. As a standard, we used a commercial *d, l*-thyroxine in

TABLE 2. NORMAL RATS

Dose thyroxine per 10 gm. per 3 days	O_2 mg. per hr. per kg.		
	before treatment	after treatment	% increase
10	1535	1576	0
10	1500	1650	10
10	1550	2000	29
15	1600	1900	18
25	1670	2000	20
25	1640	1965	20
25	1450	1980	37
25	1550	2050	32
25	1600	2250	41
28	1420	2090	47
31	1600	2260	39

crystalline form. It was administered in slightly alkaline solution which, within a 3-day limit, was freshly prepared.

In comparison the following preparations were investigated.

a), THYROID GLOBULIN, extracted from pig thyroids. This contained 0.565% total iodine; 0.14% thyroxine iodine was found by the method of Leland and Foster. Since the identical value was obtained by Blau's procedure (9), both methods were considered to be equivalent. The determined value was corrected in accordance with the estimated loss of 15% by this method, giving 0.165% thyroxine iodine (=29.1% of total iodine) or 0.25% thyroxine. By splitting the thyroid globulin by alkaline hydrolysis two preparations were derived from it.

b), AN ACID-SOLUBLE FRACTION CONTAINING 0.26% TOTAL IODINE.

c), AN ACID-INSOLUBLE FRACTION OF 4.24% IODINE. The former, supposed to contain essentially the diiodotyrosine fraction, assayed still 0.0085% thyroxine iodine or 0.01% corrected, corresponding to 0.015% thyroxine. The insoluble fraction was supposed to contain the thyroxine compound. It assayed 2.18% thyroxine iodine, corrected (=51.4%) or 3.33% thyroxine.

d), A COMMERCIAL U.S.P. THYROID GLAND PREPARATION, CLAIMED TO BE STANDARDIZED BY THYROXINE CONTENT, BUT WITH NO INFORMATION AS TO METHOD OR STANDARD. It contained 0.2% total iodine, and 0.061% thyroxine iodine, corrected, (30.5%) equal to 0.094% thyroxine.

e), A SELECTED DRIED THYROID GLAND POWDER, OBTAINED FROM A PACKING HOUSE. This contained 0.465% total and 0.125% thyroxine iodine, corrected, (=26.9%) equivalent to 0.19% thryoxine.

For use, the acid-insoluble fraction *c*), was dissolved in a trace of alkali, like thyroxine, the acid-soluble fraction *b*), was dissolved in water. The other substances were suspended as fine powders in a tragacanth gum solution.

The various tables, with exception of 8, show uniformly that the functional relationship between dose and percentage increase of metabolism follows a curve of logarithmic type. An increase of the dose beyond the minimum that produces a measurable reaction causes initially a sharp increase in effect with relatively small changes in medication. At this stage, the variations between the individual measurements are quite considerable.

TABLE 3. THYROIDECTOMIZED RATS

Rat no.	γ daily per 10 gm.	Thyroxine O_2 mg. per hr. per kg.			Average
		Before treatment	after treatment	% increase	
19	25	1200	1750	46	46
20	12.5	1050	1620	46	46
23	10	1020	1490	45	45
24	6	1200	1700	42.5	41
25	6	1250	1740	40	
27	3	1100	1500	36	
98	3	925	1360	47	
29	3	1050	1420	35	
99	3	990	1440	45	42
30	3	1000	1390	39	
77a	3	900	1335	48	
103	3	975	1400	45	
104a	3	1000	1400	40	
28	2	1060	1450	37	
34	2	980	1350	36	33
36	2	1100	1390	27	
31	1	1220	1550	27	
37	1	970	1310	35	30
38	1	1110	1420	28	
32	0.75	1050	1310	25	
41	0.75	1100	1380	25	
52	0.75	900	1230	36	28.8
57	0.75	1130	1440	28	
39	0.75	1110	1430	29	
40	0.75	1150	1500	30	
33	0.5	1160	1280	11	
35	0.5	930	1020	10	
40	0.5	1025	1075	5	9.6
49	0.5	1120	1208	8	
54	0.5	1200	1350	12	
53	0.5	1155	1295	12	

At the other end of the curve, where it approaches a plateau, the responses are more uniform, but considerable variations in dosage produce slight differences in reactions which are nearly within the limits of error. For a comparative study, the central part of the curve (fig. 1, e–f) is the most appropriate, where the relation between dosage and response approaches a linear function. A comparison of the various products tested must be made within that range, which covers in our case a metabolic increase of 20 to 35%.

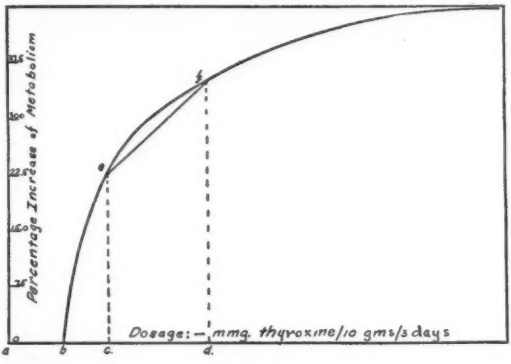

Fig. 1

In the case of normal rats, a treatment with 25 γ thyroxine gave responses generally falling within those limits; however, the individual variations were wide and the number of tests necessary to draw any statistical conclusion had to be large. The thyroidectomized rat proved to be not only far more sensitive but also gave more uniform results. An effect corresponding to 25 γ thyroxine in the normal rat was obtained with 0.75–1.0 γ in the thyroidectomized animal, showing that the latter is about 25–30 times more sensitive. The use of thyroidectomized animals permitted the selection of a more narrow range, namely, 25 to 30% average increase of metabolism as a standard reaction. Consequently, 0.75 γ thyroxine was chosen as the standard medication. If thyroxine as such were the only active constituent in thyroid material it should be expected that the metabolic activity of any substance administered at a dose of 0.75 γ thyroxine content would give the standard increase, provided, of course, that the analytical method can be relied on to give true and fairly accurate thyroxine values. This expectation had to be modified, in view of the fact that the thyroxine used was the racemic *d,l*-mixture, whereas the natural thyroxine is the *l*-compound. Admitting the possibility that the *d*-compound is absolutely inactive, as Palmer and Leland have claimed, natural thyroid material might show maximally a double potency; but at least proportionality should exist between various types of natural thyroid material. The tables show that neither is the case.

The purified globulin fraction gives the standard effect at a thyroxine value between 0.19 and 0.25 γ probably 0.21–0.22, an activity 3½ times higher than that of thyroxine.

The corresponding values of the acid-soluble fraction are: 0.45 γ thyroxine content, of the U.S.P. thyroid powder 0.3 and of the high potency thyroid powder 0.25 γ.

Calculating the total iodine instead of thyroxine as a standard of potency one finds:

0.75 γ thyroxine ≃ 0.5 γ iodine
85 γ thyroid globulin ≃ 0.48 γ iodine
3000 γ acid-soluble fraction ≃ 7.8 γ iodine
320 γ U.S.P. thyroid ≃ 0.64 γ iodine
133 γ high grade thyroid powder ≃ 0.62 γ iodine

TABLE 4. THYROIDECTOMIZED RATS

Rat no.	Dose γ per 10 gm. per 3 days	Thyroid globulin equivalent thyroxine	O_2 consumption γ/hr./kg.			Average
			Before treatment	After treatment	% increase	
89	40	0.1	950	1050	10	
40	40	0.1	1125	1235	10	
88	40	0.1	1235	1280	4	7
86	40	0.1	1100	1210	10	
85	40	0.1	1200	1240	3	
80	40	0.1	1040	1170	7	
87	50	0.125	1020	1190	17	
83	50	0.125	1030	1160	13	
82	50	0.125	1100	1320	20	
81	50	0.125	1030	1180	15	
79	50	0.125	1000	1190	19	17
77	50	0.125	925	1100	18	
76	50	0.125	1060	1230	16	
75	50	0.125	970	1190	21	
74	50	0.125	1100	1320	20	
73	50	0.125	1110	1270	14	
44	65	0.16	1010	1240	23	23
71	75	0.19	960	1150	20	
69	75	0.19	980	1250	26	
67	75	0.19	1000	1310	31	24
66	75	0.19	1080	1365	26	
70	75	0.19	1100	1310	18	
68	75	0.19	1130	1380	22	
63	100	0.25	1130	1450	28	
61	100	0.25	1050	1425	36	
58	100	0.25	940	1235	32	31
59	100	0.25	1100	1466	33	
65	100	0.25	1200	1540	28	
91	200	0.5	960	1360	43	43
92	300	0.75	975	1445	48	
96	300	0.75	1100	1610	46	
94	300	0.75	950	1400	50	46
97	300	0.75	1100	1580	44	
95	300	0.75	1170	1670	43	

The last two preparations, being in quite close agreement, might suggest that the total iodine could be used as a means of evaluating thyroid preparations against one another, as far as total thyroid material is concerned. To make such claims, a large number of thyroid preparations of varying provenience had to be tested. The acid-soluble fraction speaks strongly against that theory, but even disregarding that substance as an artifact, there remains the objection that thyroid globulin shows a 25% higher potency, and in this case the chemical handling consisted merely in a process of extracting

and precipitating with elimination of certain substances, but probably with no chemical change of the globulin compound itself.

Three or four times the optimal dose seemed to give, with little variation, an average increase of 46 to 48%. This could not be tested in the case of the acid-soluble fraction, because the doses became too large to be given conveniently. The increase of 46 to 48% returns the metabolism of the animal to that of the normal rat, and it seems that beyond that limit far larger doses of thyroid are required to bring about a hyperthyroid metabolism. This fact explains the results of Gaddum, who rejected the thyroidectomized rat

TABLE 5. THYROIDECTOMIZED RATS

Rat no.	Dose γ per 10 gm. per 3 days	Acid-soluble fraction				Average
		Content of thyroxine	O_2 consumption mg./hr./kg.			
			Before treatment	After treatment	% increase	
36b	500	0.075	920	960	0	
43	1000	0.15	1060	1120	5	0
100	1000	0.15	1050	1050	0	
101	1000	0.15	1070	1060	0	
178	2000	0.30	1185	1350	14	
105	2000	0.30	920	1060	15	
106	2000	0.30	1090	1190	9	12
109	2000	0.30	1040	1150	10	
107	2000	0.30	960	1090	13	
110	3000	0.45	900	1170	30	
147	3000	0.45	900	1160	30	
108	3000	0.45	1140	1360	30	28
111	3000	0.45	960	1290	34	
113	3000	0.45	920	1175	28	
114	3000	0.45	960	1210	26	
116	6000	0.9	1030	1410	37	
112	6000	0.9	1000	1350	35	
115	6000	0.9	1040	1310	26	31
117	6000	0.9	880	1160	32	
118	6000	0.9	1060	1350	27	
104b	10000	1.5	900	1220	36	

as a test animal for not being more sensitive than the normal rat. His doses were too high and consequently within the range of lower sensitivity. Necessarily his rats responded similarly to normal rats.

The acid-insoluble fraction, which is relatively rich in thyroxine by assay, gave rather disappointing results. Increase of dosage brought about repeatedly a decrease in effect. It can only be said that the latter is not proportionate to the thyroxine content, but considerably lower, quite contrary to expectations. Although high in thyroxine value, the preparation was probably less uniform in composition than the other material tested, being collected in small yield from various experiments of hydrolysis. It was noticed that the product is not completely soluble in alkali, but a fine residue remained suspended in the solution. This had possibly some influence on the absorption, which may have become erratic.

Recent work by Thompson and coworkers (10) showed that in patients, thyroxine given orally, either as such, as the sodium salt or as an alkaline

solution, varied widely in potency. We did not have such experience with thyroxine, although the agreement in results was better in the tests with thyroid or thyroid globulin. In the case of our acid-insoluble preparation, we

TABLE 6. THYROIDECTOMIZED RATS

Rat no.	Dose γ per 10 gm. per 3 days	Commercial Thyroid Powder U.S.P. XI				
		Content of thyroxine	O_2 consumption mg./hr./kg.			Average
			Before treatment	After treatment	% increase	
127	320	0.3	930	1170	26	
122	320	0.3	1210	1520	26	
129	320	0.3	1050	1360	30	30
126	320	0.3	1000	1350	35	
140	320	0.3	1000	1350	35	
77c	320	0.3	920	1170	27	
132	1280	1.2	950	1440	53	
131	1280	1.2	1000	1400	40	46
128	1280	1.2	980	1480	50	
137	1280	1.2	1000	1425	42.5	

are dealing with a split product high in thyroxine, which might account for a behavior akin to that reported by those authors. This, of course, still leaves the question open, why even experiments using the same solution, that is, of identical concentration and alkalinity, gave still variable readings.

TABLE 7. THYROIDECTOMIZED RATS

Rat no.	Dose γ per 10 gm. per 3 days	Thyroid Powder with 0.465% I				
		Content of thyroxine	O_2 consumption mg./hr./kg.			Average
			Before treatment	After treatment	% increase	
154	133	0.25	1165	1500	29	
155	133	0.25	960	1260	31	
156	133	0.25	930	1240	33	30
104	133	0.25	900	1200	33	
163	133	0.25	970	1215	25	
152	167	0.32	880	1200	36	
148	167	0.32	1000	1440	44	41
151	167	0.32	1040	1500	44	
164	400	0.75	1150	1750	52	
166	400	0.75	1050	1475	40	48
161	400	0.75	1050	1590	52	
153	668	1.28	940	1500	58	

The question might be brought up, whether the calculation on percentage increase of oxygen consumption is an adequate means of measuring the calorigenic effect. Theoretically it could be visualized that a given quantity of hormone takes care of a definite quantity of oxygen, as a similar attempt has been made to ascribe to a U of insulin the combustion of a certain quantity of glucose. The fact that dose increase at high levels causes little or no increase of oxygen consumption speaks against this possibility. Besides,

if this were the case, rats with an initial metabolism of 900 should show uniformly a higher percentage increase than rats with 1100–1200 initial values. The tables do not agree with such anticipation. Perhaps it will be possible, with accumulation of more data and improvement of technic, to work out a mathematical formula that will give a more adequate interpretation of the relation between cause and effect in that connection. It will be

TABLE 8. THYROIDECTOMIZED RATS

Rat no.	Dose γ per 10 gm. per 3 days	Acid-insoluble (thyroxine) fraction				Average
		Content of thyroxine	O_2 consumption mg./hr./kg.			
			Before treatment	After treatment	% increase	
119	7.5	0.25	1050	1100	5	5
125	22.5	0.75	1000	1000	0	8
124	22.5	0.75	910	1050	16	
121	30.0	1.0	920	1100	20	
133	30.0	1.0	1150	1310	13	18
113	30.0	1.0	1120	1345	20	
136	30.0	1.0	1020	1230	21	
135	37.5	1.25	950	1200	29	
138	37.5	1.25	1020	1370	34	
141	37.5	1.25	1120	1410	26	26
142	37.5	1.25	940	1140	21	
143	37.5	1.25	900	1110	23	
158	40.0	1.33	900	1080	20	
160	40.0	1.33	990	1135	14	
162	40.0	1.33	1040	1240	19	17
165	40.0	1.33	1100	1246	13	
147	40.0	1.33	930	1095	18	
170	60.0	2.00	895	1050	17	
167	60.0	2.00	990	1220	23	23
169	60.0	2.00	1000	1275	27.5	
174	80.0	2.67	1050	1375	31	19
168	80.0	2.67	1000	1080	8	
145	90.0	3.0	960	1230	28	28
144	150.0	5.0	980	1280	30	
150	150.0	5.0	1100	1500	36	29
146	150.0	5.0	1075	1290	20	

noticed also that the logarithmic curves if sketched for each product separately do not run absolutely parallel.

In picturing such relationship in connection with a logarithmic curve it must be noticed that the mathematical curve gives a value zero at dose 'one' (point b), being negative at fractions. It might be interesting to consider the meaning of the stretch a–b on the abscissa. Negative values are obviously meaningless.

In complete absence of a physiological agent, in our case thyroid hormone, there is necessarily a subthreshold level for medication too small to cause even a minimum of effect. This should correspond to a–b. At the moment, this minimum is passed, a sharply increasing effect sets in corresponding to the steep ascending part of the curve. The dose, consequently, should be calculated as consisting of two parts (a–b) and (b–c), where b–c is the really active part. Since a–b has the value 1, it might properly be named the

natural unit; its determination, however, would probably meet with considerable difficulties. The consideration of this possibility, that a subthreshold quantity of hormone might remain for a longer period within the organism, even when the metabolism has returned to the pre-treatment level, induced us not to use a rat over again after the first treatment, and to allow a lapse of at least 4 weeks after thyroidectomy.

SUMMARY

The oxygen consumption of normal and thyroidectomized rats was determined in a closed circuit metabolic apparatus before and after treatment with thyroid material. Medication was given by stomach tube on 3 consecutive days and the change of metabolism observed on the 5th day. The thyroidectomized rat was found to be 25 to 30 times more sensitive than the normal rat and to give more uniform results. An increase of 25 to 30% in oxygen consumption, most suitable for comparison, was obtained in the thyroidectomized rats by giving on each of these days 0.75 γ thyroxine for each 10 gm. body weight. The other substances on test were analyzed for their thyroxine content by the Leland-Foster method, and fed in proportionate doses. The following quantities of thyroxine, given in various compounds, produced a corresponding increase in metabolism: Thyroid globulin, of 0.565% I and 0.25% thyroxine: 0.21–0.22 mg.; an acid-soluble hydrolysate, 0.262 I and 0.015% thyroxine: 0.45 mg.; U.S.P. thyroid powder, 0.2% I and 0.094% thyroxine: 0.3 mg.; thyroid powder of 0.465% I and 0.19% thyroxine: 0.25 mg. An acid-insoluble hydrolysate of thyroid globulin, 4.24% I and 3.33% thyroxine gave inconsistent results. At approximately three times the dosage, as given for comparison, the metabolism of the thyroidectomized rats approaches that of the normal animal (45 to 50% increase) and considerably larger doses are necessary to cause further increase in metabolism. It appears that neither iodine alone nor thyroxine, as ascertained by chemical assay, give a uniform means of estimating metabolic activity of thyroid material. The chemical linkage within the molecule seems to be an important factor of influence in the physiologic action.

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THE INFLUENCE OF DESICCATED THYROID, THYROID CONCENTRATE AND THYROXINE ON THE OXYGEN CONSUMPTION OF THE GUINEA PIG

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ALTHOUGH THE physiological effect of thyroxine compares qualitatively with that of the whole thyroid, it has been impossible to associate the total activity of the thyroid with the amount of thyroxine in the gland or the maximum amount of thyroxine it could contain on the basis of the acid-insoluble fraction. It is difficult as well as inaccurate to compare the activity of the thyroid gland with thyroxine since the gland can be administered only by mouth while thyroxine is most effective when injected. Harington and Salter (1) and later Harington and Randall (2) have shown by the enzymatic hydrolysis of thyroglobulin that thyroxine and diiodotyrosine occur as constituent amino acids in the protein of the thyroid.

Except for a trace of inorganic iodine all of the iodine of the thyroid can be accounted for by the two compounds thyroxine and diiodotyrosine. Mild alkaline hydrolysis of thyroprotein produces a fraction containing all of the thyroxine. The whole of the physiological activity is concentrated in this fraction. The possibility that the inactive fraction containing diiodotyrosine could exist combined with thyroxine (not necessarily directly) in the thyroid in active form always exists.

Inasmuch as thyroxine represents about 50% of the total iodine and it is assumed that all of the activity is due to thyroxine, it should be possible to prepare from the gland a thyroxine-containing complex which, on the basis of its iodine content, should be twice as active as the original gland. Enzymatic degradation products of thyroglobulin have been prepared with the hope of obtaining such an active peptide of thyroxine (3). These investigators have shown rather conclusively that the peptide and whole thyroid gland based on iodine equivalents have about the same physiological properties when administered orally.

In 1931, Blum (4) reported that he was able to isolate from fresh glands, an iodine-containing protein which, when compared to whole thyroid or

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thyroxine, was many times (10 to 35) as active in its ability to increase the B.M.R. This product is known commercially as thyractin or elityran.

We have prepared a protein from fresh hog thyroid glands according to Blum's directions. Briefly the method consists of extracting freshly ground hog thyroid with water and subjecting this aqueous extract to treatment with various concentrations of saturated ammonium sulfate. The two fractions precipitated when the concentration represents 60 and 80 parts by volume of saturated ammonium sulfate respectively per 100 parts by volume of original thyroid solution are the active fractions and are designated as fractions 1 and 2. These fractions are dissolved in water, dialized and finally evaporated in vacuo to a dry powder. A study was then made comparing the ability of Blum's concentrate (prepared by us), desiccated thyroid and thyroxine to increase the O_2 consumption in the guinea pig.

EXPERIMENTAL

Guinea pigs weighing between 250 and 450 gm. were placed on a uniform diet consisting of a mixture of 1 part bran and 10 parts rolled oats and 0.1 gm. NaCl. They were fed that amount of this mixture daily which maintained their weights at a constant level. This amount ranged from 13 to 19 gm. In addition, each animal was given 15 gm. of lettuce daily. Once the amount needed for this maintenance of constant weight was determined, the animal received the same quantity of food throughout the course of the experiment. In some cases the animals stopped eating. Thus a loss in weight and a decrease in metabolism arose due to inanition. Such animals were discarded.

When the weight remained constant for 6 or more consecutive days, the O_2 consumption of each animal was determined. Food cups were removed 8 to 10 hours before such determinations were made. The animal was placed in a chamber devised by Richards and Collison (5) for the determination of the O_2 consumption of small animals. These determinations were made on alternate days at least 3 times in order to determine a normal for each animal.

The materials to be studied were administered in capsules by mouth. The O_2 consumption was determined on alternate days for 7 days. On the 8th day no supplement was added and the final weight of the animal was determined at the end of that day. This weight represented the final weight for the experimental period. The body weight and amount of food consumed daily were recorded. Total iodine determinations were made on the supplements by the method of Davis and Curtis (6) with some slight modifications.

We found that by calculating the O_2 consumption on the basis of surface area that the data could be more accurately compared. The surface area was calculated by the method given by Meeh (7). The value in the table for O_2 consumption are hundredths cc. of oxygen per sq. cm. of surface area. The sample of desiccated thyroid used (0.4552% iodine) was arbitrarily designated as one equivalent of iodine. Originally 60 mg. of desiccated thyroid were fed to one group of animals. Another group was fed 50.7 mg. of Blum's concentrate (one equivalent of iodine).

The average increase in O_2 consumption in both groups was 81 to 85. It was possible that the amount administered exceeded the quantity which would give a maximum increase in O_2 consumption so that any differences between the two preparations existed, these differences would be masked.

TABLE 1

Iodine Equivalents	Desiccated thyroid			Blum's concentrate		
	No. of animals	Ave. % loss in wt.	% Increase in O_2 consumption	No. of animals	Ave. % loss in wt.	% Increase in O_2 consumption
1	4	26, 27, 20, 19 — 23	119, 98, 99, 22 — 85	5	16, 23, 28, 18, 30 — 23	92, 84, 53, 99, 75 — 81
1/2	3	14, 30, 19 — 21	81, 59, 38 — 59	4	13, 15, 23, 15 — 16	63, 118, 113, 63 — 94
1/4	2	18, 11 — 14	33, 43 — 38	3	17, 11, 13 — 13	51, 61, 103 — 72
1/8	3	19, 8, 11 — 13	74, 29, 19 — 40	3	15, 20, 30 — 22	29, 50, 22 — 33
1/16	3	16, 11, 11 — 13	39, 8, 20 — 22	3	6, 12, 21 — 13	46, 51, 46 — 48
1/32	3	13, 10, 5 — 9	7, 45, 13 — 22	3	5, 6, 0 — 3	24, 20, 23 — 22
1/64	3	11, 4, 2 — 6	18, 0, 10 — 9	4	20, 0, 4, 5 — 7	30, 6, 13, 16 — 16
		Thyroxine (Na salt)				
1 1/2	3	17, 23, 22 — 21	53, 57, 57 — 56			
3/4	3	12, 21, 23 — 19	71, 37, 55 — 54			

We therefore administered (table 1) decreasing dose of both desiccated thyroid and Blum's concentrate ranging from ½ to 1/64 of an equivalent of iodine. The data clearly indicate that there were no marked differences between these substances in their ability to increase the oxygen consumption when administered in equivalents of iodine.

Only two series of animals were studied with thyroxine which was administered as the sodium salt. The differences were not striking enough to

warrant a study of the effect of smaller doses. Reports of other studies have shown that thyroxine is less effective when administered orally than when injected. Since our studies were concerned with oral administration, we did not continue the thyroxine studies.

The table represents the results of every experimental animal used except those that were discarded earlier due to failure to consume the diet.

SUMMARY

A thyroid concentrate prepared in accordance with the published method of Blum was compared with desiccated thyroid and thyroxine in its ability to increase the O_2 consumption of the guinea pig. Blum's concentrate has approximately the same effect as desiccated thyroid in raising the basal metabolism when given in the same equivalents of iodine. No evidence was obtained which would indicate that Blum's concentrate is superior to desiccated thyroid in increasing the O_2 consumption.

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INFLUENCE OF THYROID ON EGG PRODUCTION[1]

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GRAHAM (1) reported that thyroidectomy tends to depress and thryroid administration to elevate the rate of milk production of dairy cows. Benoit and Aron (2) reported that the testes of White Leghorns rapidly decreased in size following thyroidectomy. Losses were as great as 80% of the testis size in 11 days and 90% in 20 days. Similar results were obtained with drakes.

Repetto (3) was unable to detect histological changes in testes or ovaries of dogs following thyroidectomy. Zelesky and Wells (4) reported that of 11 thyroidectomized, thirteen-lined ground squirrels (*Citellus tridecemlineatus*) without thyroid tissue detectable by histological examination, 7 exhibited at the normal breeding season an infantile condition as regards both spermatogenesis and testis hormone production, and one reached a state of very low sexual activity. However, 3 were apparently normal in regard to sexual development, but, as is pointed out by the authors, thyroid tissue may have been present in these 3 even though it was not detectable.

Fishburn and Cunningham (5) have reported that after thyroidectomy the reproductive cycles of rats are lengthened. The basal heart rate of thyroidectomized rats of 222 ± 16 beats per minute was raised to the normal level of 300 ± 20 with 0.04 mg. of thyroxin per rat daily. These authors believe that this amount of thyroxin may approximate the daily thryoxin production of their rats (weights of rats were not given).

Taylor and Burmester (6) (private communication) have found the egg production of thyroidectomized hens to be of the order of 26 to 36% of that of normal fowls during the laying year. Two groups, one of White Leghorn hens and one of Black Minorca × Rhode Island Red, were studied. The influence of thyroid on feather growth of fowls has received attention (7, 8, 9, 10, 11).

METHODS AND MATERIALS

The purpose of this research is the determination of possible relationships between the thyroid and egg production in fowls. Influences were determined, first of thyroidectomy on egg production, and second of thyroxin injection on egg production of thyroidectomized hens.

Twenty-two White Leghorn hens were thyroidectomized under light ether anesthesia. As a safeguard against unduly low calcium level of the blood 5% of

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calcium lactate was dissolved in the water supplied the operated birds. Usually part or all of the parathyroid tissue closely associated with the thyroid was removed, but since the fowl ordinarily seems to possess accessory parathyroid bodies the calcium ration may not have been necessary. After the operation each of the birds was kept in a separate battery compartment. After thyroidectomy the hens quickly assumed the general appearance of non-laying fowls.

Approximately two months after the operation the 17 surviving hens were divided into 2 groups, one of which was to receive thyroxin, and the other to serve as a thyroidectomized control group. In general the former group was made up of birds showing somewhat greater decreases in metabolic rate and comparatively greater decreases in egg production than the control group. Thus the division was made in such a way as to decrease the chances of success (that is the birds which seemed least likely to resume laying were placed in the group which was to receive thyroxin injections). Very soon after thyroxin replacement therapy was begun the thyroidectomized hens so treated had the appearance of healthy laying fowls. Four of the thyroidectomized hens which did not show as marked declines in egg production as did the others were divided equally between the thyroxin injected, and the non-injected, group. The general appearance and behavior of these 4 hens led to the belief that in these cases thyroidectomy had been less complete than it had been in the group as a whole.

The apparatus with which the respiratory exchange was measured consists of a constant temperature cabinet in which are placed 4 independent respiration chambers each connected to an oxygen burette and a battery for the absorption of CO_2. Oxygen consumption is calculated periodically from burette readings, while CO_2 production is determined by titration. Constant pressure of 2 mm. of water is maintained in each system of chamber, burette, and battery by means of a Mariotte bottle from which water runs into each burette. Alcohol tests of the apparatus yielded a mean R. Q. of 0.67 with an error of less than 2%.

RESULTS

Table 1 shows egg production of the thyroidectomized hens (groups 1 and 2) and of a normal group of birds during the various periods of the experiment. The records of the 4 hens least affected by the operation are not included in the table, as it happened that the ratio between egg production of the thyroxin-injected and the non-injected thyroidectomized groups was not materially influenced by the deletion of these data, whereas if they had been included a false conclusion might have been reached as to the effectiveness of thyroxin in restoring the production of the operated birds. After the operation egg production of group 1 dropped from 3.77 ± 0.39 to 0.42 ± 0.18, and of group 2 from 3.50 ± 0.44 to 0.81 ± 0.32 eggs per hen per week (table 1). Production of the uninjected group remained low throughout the experiment. Thyroxin solution was injected twice a week intramuscularly at an initial level of 0.93 mg. per $kg.^{0.73}$, per week, of body weight.[2] After 9 days at the original level the thyroxin dosage was doubled, and kept at the higher level during the remainder of the experiment because the metabolic rate seemed to remain below the preoperative level when thyroxin was in

[2] The use of a power of the body weight as an expression of body size has been discussed by Kleiber (12), by Brody and Procter (13), and by Brody, Procter, and Ashworth (14).

injected in the original amount. Even with the increased dosage the metabolic rate appeared to be definitely below the preoperative level during July (table 2).

As shown by table 1 thyroidectomized hens produced between 3.8 and 4.5 times as many eggs (per hen per week) while receiving the thyroxin treatment as they had produced during the period of approximately two months between the time of the operation and the first thyroxin injection.

TABLE 1. EFFECT OF THYROIDECTOMY ON EGG PRODUCTION OF HENS, AND INFLUENCE OF THYROXIN ON EGG PRODUCTION OF THYROIDECTOMIZED FOWLS

	Group 1 Thyroidectomized fowls injected with thyroxin			Group 2 Thyroidectomized fowls not injected with thyroxin		Normal fowls
Number of birds used		7			6	17
	Production eggs/hen/week	% of production of normal fowls	Weekly dosage	Production eggs/hen/week	% of production of normal fowls	Production eggs/hen/week
Production during months prior to operation. March 23 to April 22 inc.	3.77±0.39[1]	—	0	3.50±0.44	—	4.31±0.17
Production during period between operation and 1st thyroxin injection. April 23 to June 21 inc.	0.42±0.18	10.2	0	0.81±0.32	19.7	4.12±0.21
Production during period June 22 to 30 inc. Thyroxin 1st given on June 21	1.88±0.41	43.5	0.93 mg. thyroxin per kg. $^{0.73}$	0.13	—	4.33±0.20
Production during July	1.77±0.41	50.6	1.86 mg. thyroxin per kg. $^{0.73}$	0.75±0.21	21.3	3.52±0.23
Production during August	1.58±0.36	60.1	1.86 mg. thyroxin per kg. $^{0.73}$	0.75±0.21	28.5	2.63±0.32

[1] In this paper the figures following the ± signs represent standard errors of means.

Included in the injected group is one hen which failed to lay until September, which month marked the end of the laying season for the other birds, and consequently the end of this phase of the experiment.

Data on fasting heat production are given in table 2. As fasts occurring at intervals of less than a month might have adversely affected egg production, it was necessary to limit the number of determinations of fasting heat production and therefore these data do not give as definite a picture of the rate of heat production during fast as might be desired. However, the preoperative rate of heat production, which was 3.27 ± 0.11 Cal./kg.$^{0.73}$ in the case of group 1, declined after thyroidectomy to 2.81 ± 0.09 Cal/kg.$^{0.73}$, it

tended to be restored to the original level by the thyroxin injections, and if it reached the original level it is probable that that level was not greatly exceeded during the course of the experiment. During the last month of the experiment heat production during fast was 3.09 ± 0.18 Cal,/kg.$^{0.73}$; this was the highest level of fasting heat production recorded for the thyroidectomized birds. Practically no activity of the fowls was detected while the metabolic

TABLE 2. RATE OF HEAT PRODUCTION WHILE FASTING, AND BODY WEIGHT, AS INFLUENCED BY THYROIDECTOMY AND THYROXIN TREATMENT OF HENS AFTER THYROIDECTOMY

During fast of 24–28 hours	During month prior to operation March 23 to April 22 inc.	Period between time of operation and time of first injection April 23 to June 21 inc.	Period of thyroxin injection at first level. June 22 to 30 inc.		Period of thyroxin injection at second level			
					July		August	
				Weekly dosage. Thyroxin /kg.$^{0.73}$		Weekly dosage. Thyroxin /kg.$^{0.73}$		Weekly dosage. Thyroxin /kg.$^{0.73}$
Mean rate of fasting heat production	*Calories* /kg.$^{0.73}$	*Calories* /kg.$^{0.73}$	*Calories* /kg.$^{0.73}$	mg.	*Calories* /kg.$^{0.73}$	mg.	*Calories* /kg.$^{0.73}$	mg.
Group 1 Injected fowls	3.27 ± 0.11	2.81 ± 0.09	3.02 ± 0.10	0.93	2.89 ± 0.08	1.86	3.09 ± 0.18	1.86
Group 2 Non-injected fowls	3.03 ± 0.05	2.85 ± 0.18	—	0	2.98 ± 0.17	0	2.80 ± 0.09	0
Mean body weight	kg.	kg.	kg.	mg.	kg.	mg.	kg.	mg.
Group 1 Injected fowls	1.56 ± 0.06	1.42 ± 0.05	1.48 ± 0.10	0.93	1.47 ± 0.08	1.86	1.59 ± 0.10	1.86
Group 2 Non-injected fowls	1.51 ± 0.10	1.49 ± 0.13	—	0	1.43 ± 0.05	0	1.62 ± 0.06	0

rates were being determined. Records of the 4 hens not included in table 1 are likewise not included in table 2; if they had been included the data would have remained substantially unchanged. Mean body weights of the two groups of operated hens determined at various times during the experiment are given in table 2.

DISCUSSION

From the results reported herein the influence of thyroidectomy and thyroxin administration would seem to influence egg production in a manner analogous to the effects on milk secretion of thyroidectomy and thyroid feeding as reported by Graham (1). In each case a hormone appears to accelerate the tempo of life which is manifested as an acceleration in the rate of milk secretion in one case, and of egg production in the other.

Histological data on possible regeneration of thyroid tissue in the hens cannot be included in this report as it is desired that the birds be retained for further experimentation.

SUMMARY

Thyroidectomy resulted in a decrease of egg production of a group of 7 White Leghorn hens from 3.77 ± 0.39 to 0.42 ± 0.18 eggs per hen per week. When 0.93 mg. of thyroxin per kg.$^{0.73}$ was injected weekly, the egg produc-

tion increased to approximately 40% of that of a group of normal fowls. When the original thyroxin dosage was doubled the egg production of the injected thyroidectomized group increased to 50% and later to 60% of that of the normal group. Egg production of a group of 6 thyroidectomized, non-injected fowls remained low throughout the experiment. Thyroxin, in the dosages used, partially restored the rate of fasting heat production.

Thryoxin tends to restore egg production of thyroidectomized fowls in a manner analogous to the influence of thyroid in restoring milk production of thyroidectomized cows as reported by Graham.

The present data on the influence of thyroidectomy on egg production confirm the findings of Taylor and Burmester. In addition it is reported here for the first time, that injected thyroxin tends to restore egg production of thyroidectomized fowls.

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THE EFFECT OF TESTOSTERONE PROPIONATE ON THE URINARY EXCRETION OF ANDROGENS AND ESTROGENS IN EUNUCHOIDISM[1,2,3]

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WHILE THE normal pattern of androgen and estrogen excretion by the human kidney is gradually becoming clearer, the sources of the various constituents are still in part obscure and the amounts of testis secretion required to produce normal amounts of biologically active excretion products are not well known.

A few studies on recovery of injected androgens in man are available. Kochakian (1) administered urinary extracts to one normal and to one castrate man. In the normal, injections of 11 to 17 capon units (his own unit) on two occasions caused no apparent increase in androgen excretion in the urine. In the castrate the injection of 30 to 40 capon units per day for 10 days was followed by a recovery of 3% of the injected activity from the urine passed during this period. Callow (2) reports the assays of Greenwood, using the capon's comb, on the urinary excretion of androgens by 17 men with prostatic hypertrophy, receiving from 10 to 100 mg. of testosterone propionate weekly. Significant increases occurred in only 2 patients, who received 50 and 100 mg. respectively, the values in these rising from 15 to about 50 I.U. during treatment. In two eunuchs one receiving 70 and one 150 mg. of testosterone propionate, he recovered 'some 16 per cent of the administered activity' in the first and a 'still lower percentage' in the second. Callow writes discouragingly that:

> In general, the destruction and inactivation of male hormone in the body after absorption is so intense, and the yield of administered hormone recovered in the urine is so small that the present method of urinary androgen assay is unlikely to give results which have any significance in respect to the career of male hormone in the body.

McCullagh, Rumsey, and Cuyler have, however, briefly reported conclusions of a different tenor (3) and through the kindness of Dr. McCullagh we have been able to read the complete manuscript. They report the results of 102 assays for urinary androgens in 14 cases of hypogonadism. Doses of testosterone propionate ranging from 5 to 100 mg. were given in single injections and the androgen excretion followed for 1 to 6 days. They summarize their findings on hypogonads as follows:

> It appears from these observations that in cases of frank hypogonadism, 5 to 10 mg. doses may be expected to cause a rise in urinary androgens toward but not within normal range. Such a rise is maintained for little more than one day. Larger doses raise these levels further and maintain them within normal range for longer periods depending upon the size of the dose given. If the total quantity of

[1] These investigations were supported in part by grants from the Rockefeller Foundation.
[2] We are indebted to Drs. Gregory Stragnell, Erwin Schwenk, and Max Gilbert of the Schering Corporation for the testosterone propionate.
[3] The spectrographic examination was carried out by Drs. T. R. Hogness and A. E. Sidwell of the Department of Chemistry, The University of Chicago.

androgen excreted is calculated in terms of androsterone, it appears that in these cases amounts varying between approximately 20% and 70% of the injected quantities can be accounted for.

Dorfman and Hamilton (4) have recently made numerous spot assays before, during, and after the treatment of a 35-year-old man with one intra-abdominal testis with 20 mg. of testosterone propionate per day for 30 days. From an average initial value (chick-comb assay) of 13 I.U. per day the androgens rose and varied between 47 and 97 I.U. per day during treatment and had fallen to the control level by the 30th day after stopping treatment. These authors estimate 6.2% recovery if the excreted androgen be testosterone, 41% if it be androsterone, 124% if it be dehydroandrosterone, 62.4% if it be divided equally between them, taking the activity of 0.3 mg. of dehydroandrosterone as equalling that of 0.1 mg. of androsterone. These authors also report enormous increases (to 500 I.U. per day) in urinary androgens after daily oral administration of 60 mg. of testosterone propionate to this same patient and to a eunuchoid without clinical response to treatment, and concluded therefore that such material is absorbed and excreted so rapidly that no influence in the organism is possible. This conclusion is apparently based on the subjective experiences of their patients and accordingly requires confirmation.

Kochakian (1) injected castrate dogs with 125 to 400 capon units (Kochakian's unit) of urinary extract, during periods of 1 to 3 days, and recovered from 0 to 5% of the injected activity in the urine in 4 days. Ninety-six to 126 capon units of androstenedione (which has the same activity as androsterone) per day, given to each of 2 castrate dogs for 3 days, yielded 2 and 4% urinary recovery in 7 days. Injection of 3 and 5 capon units per day for 19 to 22 days into 2 rabbits with testicular tumors resulted in the recovery of 3 to 6% of the activity in the urine during this time.

The possibility of the conversion of androgens into estrogenic substances in the body has been considered by many investigators. Zondek (5) discovered that the stallion excretes tremendous quantities of estrogens in the urine and suggested that this indicates the metabolic pathway for the testis hormone in this animal. The somewhat anomalous properties of certain androgens in producing such reactions as breast and uterine enlargement and estrus have raised the same questions; Nelson and Merckel (6), for example, state, "the marked tendency of cisandrostenediol and dehydroandrosterone to produce periods of estrus in normal female rats may be due to their conversion by the ovary to an estrogenic substance."

Steinach, Kun, and Peczenik (7a) reported an increase in urinary estrogens in normal and castrate male rats after the administration of urinary extracts and of androsterone and Steinach and Kun (7b) later confirmed this in men. They injected men of various ages with 50 mg. doses of testosterone propionate 3 times weekly until 1000 mg. had been given. Only the morning sample of urine was collected. This urine was concentrated to one-fifth volume under vacuum and extracted by shaking twice with hot benzene, no separation of androgens and phenolic estrogens being accomplished. Pre-treatment values of urinary estrogens of 0 to 36 R.U. per liter rose during androgen administration to 67 to 110 R.U. during the first week and reached their highest value of 1200 R.U. per liter of morning urine after 7 weeks of treatment, returning to normal in 3 weeks. These high values are especially remarkable in view of the almost casual extraction conducted by shaking the urine twice with benzene and the absence of hydrolyzing procedures usually necessary to secure maximum yields of estrogens from urine. Callow (2) and Webster (8) have indicated in papers not directly concerned with this problem that they have data confirming Steinach and Kun, in eunuchs and in adolescent hypogonad males, respectively; but their results are not as yet available in detail. Kochakian (9) could establish no

such effects from urinary androgens, androstenedione, and several testosterone compounds given in adequate doses to 1 normal and 4 castrate dogs.

If such conversion of androgens into estrogens in man is a physiological phenomenon, the long puzzling question of the source of urinary estrogens in adult men may be at least in part solved. These estrogens pass into the phenolic fraction (10) when this is separated by solubility in sodium hydroxide from the androgen fraction of urine extracts and hence cannot be any known androgen. Dorfman, Gallagher, and Koch (11) observed that the action of the estrogenic material from male urine on the rat uterus was identical with that of theelin (estrone) and unlike that of theelol (estriol). Dingemanse, Laqueur, and Mühlbock (12) have recently crystallized estrone from male urine but hold that it is only partly responsible for the estrogenic activity of such urine, some of the activity residing in a non-ketonic fraction. Our own data on man are seen to be ambiguous in providing the sort of parallel between androgen and estrogen excretion that one would expect if a conversion of one into the other were a consistent occurrence in the body. The phenolic estrogens of normal male urine, ranging from the equivalents of some 2 to 29 γ of estrone per day, did not vary in parallel with the androgens, during the 6-week observations on 4 normal young men examined in this laboratory (10). The seven eunuchoids, however, who averaged a third of the normal amount of androgens, excreted an average of only 1.8 γ of estrone equivalents per day as compared to the average normal of 10 (13).

As the influence of administered testosterone on the excretion of urinary androgens and estrogens is still uncertain, opportunity was taken in the course of certain metabolic studies (14) on hypogonad men to examine these points. The daily intramuscular injection of 25 mg. of testosterone propionate used was sufficient to cause enlargement of the penis and of the prostate, to increase the frequency of erections and in protracted experiments to increase the sexual hair and deepen the voice in such patients, in our experience (15) and in that of others (8, 16, 17). In the metabolic observations made in parallel there occurred a striking retention of nitrogen, sodium, and usually of chloride, with gains in body weight due largely to water held in association with the nitrogen and salts, so that comparisons may be made between the excretion of the biologically active substances and the effects of the hormone in the organism.

METHODS

Complete urine collections were made on each patient including a control period of 7 to 13 days before the injections and a period of 8 to 12 days after treatment ceased. The urines in *F. R.* and *M. D.* were collected without added preservative, in *N. D.* and *H. S.*, 5 cc. of concentrated sulfuric acid was added to each gallon container. In all 4 cases the urine was hydrolyzed and extracted as soon as possible, always within 3 weeks after the start of the collection period, and usually sooner. The urine of *H. S.* was kept in the refrigerator until hydrolyzed, the others largely in the refrigerator, but also a few days at room temperature. Control experiments have demonstrated that no loss in activity is observed after 3 weeks in the refrigerator. We agree with Callow (2) in observing a considerable loss of activity after prolonged standing at room temperature, amounting in our experiments to 40% after 3 months.

After the removal of small portions for the chemical analyses (15) the successive 24-hour samples were combined into 5 to 13-day lots, one-tenth the volume of concentrated commercial hydrochloric acid added, and the acidified urine boiled for 15 minutes over a free flame thus hydrolyzing the conjugated forms in which the androgens normally appear (18) and avoiding the losses consequent upon prolonged boiling

(2, 19, 26). The urine was then extracted with benzene in a continuous extractor (10) in which each volume of urine comes in intimate contact with 12 volumes of benzene. Two and one-half liters of urine can be completely extracted in 4 hours. The benzene-soluble material was shaken with sodium bicarbonate to remove inert substances and then with sodium hydroxide to separate the phenolic estrogens. Non-phenolic androgens, some of which may have estrogenic properties are thus excluded from the alkali-soluble fraction. The neutral or androgenic fraction was dissolved in olive oil and assayed by the capon method of Gallagher and Koch (10). Seven capons were used on each sample and a standard preparation was always run in parallel.

The estrogenic fractions were assayed on spayed rats by an unpublished modification of the Gustavson-D'Amour method (20). Each preparation is injected into 20 spayed rats, given in one dose in 0.25 cc. of sesame oil, each rat having been primed 48 hours previously with 1 γ of estrone. Vaginal smears are taken after 48 and 52 hours. The number of rats showing full estrous smears at either reading is determined for each preparation. Two groups of 20 rats each, receiving 0.5 and 0.75 γ of estrone are run as controls in parallel in each assay. A standard curve was established with 100 rats run on doses of 0.0, 0.25, 0.50, 0.75, and 1.00 γ of estrone on 10 successive weeks. Each point on this curve represents 10 assays with 20 animals on each dose level. Between the points for 0.25 and 0.75 γ of estrone, the relation between gamma of estrone injected and per cent of rats in estrus is practically a straight-line function. This relationship is utilized as follows: the per cent of estrus observed on an unknown is multiplied by the value obtained by dividing the per cent of estrus on the standard curve for the 0.5 γ estrone by the per cent of estrus on 0.5 γ of estrone observed in parallel with the unknown. This correction raises or lowers the observed response to the slope of the standard curve and the amount of estrone in each unknown can then be read off. This correction is applied in order to minimize variations in sensitivity observed in the colony from week to week. Reference to a standard curve gives the statistical benefits of large numbers of animals while the use of 2 control solutions with each set of assays gives a constant check on the sensitivity and accuracy of the method. In an unselected series of 42 weekly assays carried out on this basis 37 gave a higher percentage of estrous response in the group receiving the 0.75 γ standard than in the 0.50 γ group. In 3 assays the lower dose gave the higher response. In 2 assays neither standard produced estrus. The rats used for these 2 assays were old and were subsequently discarded. In the 37 assays where the response to the standards was in the proper order, the per cent of rats in estrus produced by the 0.50 γ control solution varied from 0 to 58% with an average of 25%, on the 0.75 γ solution the response varied from 20 to 80% with an average of 46%, or an average difference of 21% between the two standards. The limit of accuracy of the method is probably 0.1 γ of estrone within the range discussed and the estrogen values reported in this paper are probably accurate within 30%.

DISCUSSION

The androgen and estrogen excretion for each patient, expressed in I.U. of androgens (1 I.U. equals the activity of 0.1 mg. of androsterone) and gamma equivalents of estrone are shown in figures 1, 2, 3, and 4, together with the data on urinary nitrogen and body weight previously described (14). In all instances the values for both androgens and estrogens rose from the subnormal base-line levels immediately after starting testosterone propionate, reaching their highest points during the second week in 2 individuals and

then fell precipitously when treatment was stopped. In 3 of 4 patients, the estrogens declined less rapidly than did the androgens during the offset periods.

The biological effects of testosterone propionate, as exemplified here by the retention of nitrogen and increased body weight become manifest during the period of increased androgen and estrogen excretion and decline in parallel with the declining hormone excretion during recovery. While in 3 individuals (*M. D.*, *N. D.*, and *F. R.*) there was no precise correspondence between the degree of nitrogen and water retention, on the one hand, and

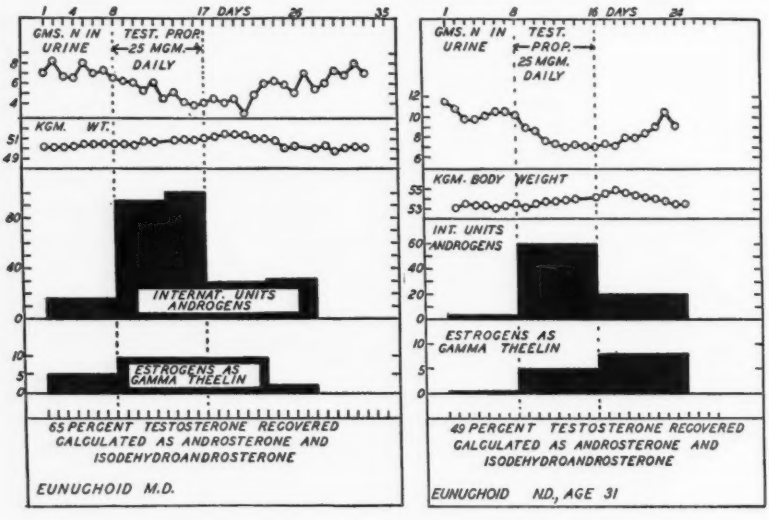
1 4 8 17 DAYS 26 35
GMS. N IN URINE
TEST. PROP. ←25 MGM.→ DAILY
KGM. WT.
INTERNAT. UNITS ANDROGENS
ESTROGENS AS GAMMA THEELIN
65 PERCENT TESTOSTERONE RECOVERED CALCULATED AS ANDROSTERONE AND ISODEHYDROANDROSTERONE
EUNUCHOID M.D.
1 8 16 DAYS 24
GMS. N IN URINE
TEST. PROP. 25 MGM. DAILY
KGM. BODY WEIGHT
INT. UNITS ANDROGENS
ESTROGENS AS GAMMA THEELIN
49 PERCENT TESTOSTERONE RECOVERED CALCULATED AS ANDROSTERONE AND ISODEHYDROANDROSTERONE
EUNUCHOID ND., AGE 31

Fig. 1 and Fig. 2

the amounts of androgens and estrogens excreted on the other hand, *H. S.* showed differences from these 3 that are of particular interest. His hypogonadism was due to the injury of the pituitary body by a craniopharyngioma, confirmed at autopsy, while in the others the etiology was unknown, with certainly no evidences of gross pituitary damage. The androgen and estrogen excretion curves in *H. S.* differ in that the rise in androgens is much smaller and that there is an immediate drop to pre-treatment levels after stopping the injections in contrast to the more gradual return otherwise found. The smaller response to testosterone administration evident in the curve for androgen excretion is associated with a slighter degree of nitrogen retention (1.16 gm. N per day as compared to 2.12, 2.39, and 4.51) and the more rapid decline in androgen excretion during recovery is associated with a much sharper rise of nitrogen excretion. To what extent these peculiarities of *H. S.* are due to his hypopituitary state, which, of course, would have affected other tissues as well as the gonads and to what extent they are chance variations, only further work can determine.

In all 4 cases, testosterone propionate increased androgen and estrogen excretion sufficiently to bring the values within normal limits, which for men, in the experience of this laboratory, is for androgens, 22 to 132 I.U., on the basis of the 15-minute hydrolysis, giving an average of 67 I.U.; for the estrogens, the equivalents of 2 to 26 γ of estrone with an average of 10 (10). This suggests that the normal estrogens of male urine may well have their origin in large part in the metabolism of the testis hormone; thus concurring in Zondek's conception of the source of the estrogens in stallion's urine. The parallel fall in estrogens and androgens in the urine of eunuchoids

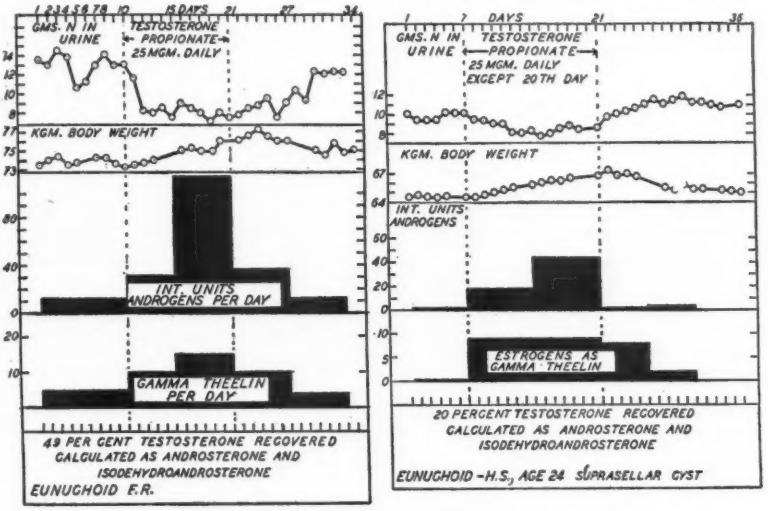
GMS. N IN URINE
TESTOSTERONE PROPIONATE 25 MGM. DAILY
KGM. BODY WEIGHT
INT. UNITS ANDROGENS PER DAY
GAMMA THEELIN PER DAY
49 PER CENT TESTOSTERONE RECOVERED CALCULATED AS ANDROSTERONE AND ISODEHYDROANDROSTERONE
EUNUCHOID F.R.
DAYS
GMS. N IN URINE
TESTOSTERONE PROPIONATE 25 MGM. DAILY EXCEPT 20TH DAY
KGM. BODY WEIGHT
INT. UNITS ANDROGENS
ESTROGENS AS GAMMA THEELIN
20 PERCENT TESTOSTERONE RECOVERED CALCULATED AS ANDROSTERONE AND ISODEHYDROANDROSTERONE
EUNUCHOID - H.S., AGE 24 SUPRASELLAR CYST

Fig. 3 and Fig. 4

as evidenced by the control values in these experiments, and in previous studies (13) is in agreement with this view and the apparently conflicting observation that the androgen-estrogen ratio in the urine of normal men (10) varied widely may possibly be accounted for by the considerable lag in estrogen excretion as compared to that of the androgens after discontinuing testosterone propionate. Thus physiological lowering of testis secretion might well be reflected later in the estrogens than in the androgens and any precise correlation between the two in any given collection period would accordingly not be expected. The continued excretion, however, of small amounts of estrogens in the urine of castrate men (3 to 4.5 γ of estrone per liter in 2 men [13], 2 to 17 M.U. per liter in 4 men [21]) points to an extragonadal origin for some of this material.

The conversion of testosterone to phenolic estrogens is, however, quantitatively very limited in man. In the case of *M. D.*, for example, the excess estrogens, calculated as estrone, represent 0.127 mg., or only 0.06% of the testosterone given; even when proper allowances are made for the sub-

stantial destruction of estrogens in the body itself the fraction of the testosterone thus transformed is minute. The pure estrogens are, however, so very active that it is quite possible that even these small amounts derived from testis secretion may be of physiological importance and thus account for the occasional breast hypertrophy produced in the eunuchoid by testosterone treatment (13, 17) and for the slight breast swellings of the normal pubescent boy (22). The fact that our values fall considerably short of those of Steinach and Kun (7b), previously mentioned, may be due to differences in extraction, assay, and unitage, or to the fact that their subjects were apparently not hypogonads.

The exact chemical nature of the androgens excreted after testosterone propionate injections is not known. Spectrographic examination of these fractions indicated that the characteristic absorption band of testosterone

TABLE 1. EXTENT OF RECOVERY OF ADMINISTERED TESTOSTERONE PROPIONATE AS URINARY ANDROGENS

Patient	Mg. of testosterone propionate	Equivalent of testosterone[1]	I.U. of excess androgens excreted	Recovery calculated as					
				Androsterone		Dehydroandrosterone		Equal wts. of androsterone and dehydroandrosterone	
				mg.[2]	%	mg.[3]	%	mg.[4]	%
M. D.	250	210	955	95.5	45.5	239	114	136	65
N. D.	225	189	649	64.9	34.3	162	86	93	49
F. R.	300	252	858	85.8	34.1	214	85	123	49
H. S.	350	296	406	40.6	13.7	101	34	58	20

[1] Equivalent weight of testosterone = weight of propionate × 0.84.
[2] 1 I.U. androgen = 0.1 mg. androsterone.
[3] 1 I.U. androgen = 0.25 mg. dehydroandrosterone.
[4] 7 I.U. androgen = 1.0 mg. urinary androgens if made up of equal weights of androsterone and dehydroandrosterone.

itself was absent. Very slight estrogenic activity was found in the non-phenolic or androgen fraction, and no androgenic activity was present in the phenolic fraction. These tests permit us to rule out the possibilities of the excretion of non-phenolic estrogens or of phenolic androgens following testosterone treatment. Since the biological activity of normal urine resides largely in the androsterone and dehydroandrosterone present, recovery calculations are presented in terms of each alone and in terms of equal amounts by weight of the two (table 1). This last is the usual situation in normal male urine according to the evidence available to date (23, 24, 25, 26). One hundred gamma of androsterone is equal in comb-growth production to 250 γ of dehydroandrosterone, according to the assays of Tschopp and Ruzicka, cited by Koch (27), and a number of determinations in this laboratory. Calculated as androsterone, from 13.7% (*H. S.*) to 45.5% (*M. D.*) of the injected testosterone was recovered during the experiment; calculated as dehydroandrosterone, from 34.3% to 114%, calculated as equal amounts by weight of the two, from 20% to 65%. It is thus seen that substantial fractions of the injected testosterone are recoverable from the urine of the eunuchoid, the only low values being in the hypopituitary *H. S.* Our average for the 3

hypogonads of unknown etiology of 54% (equal weights of androsterone and dehydroandrosterone present) is approximately the same as that of Dorfman and Hamilton (4), 62.4%, in a somewhat different experiment, and of McCullagh et al. (3), 20 to 70%, calculated as androsterone, both in hypogonads. A recalculation of Callow's data in a eunuch (2), if we understand them correctly, gives a recovery value of 63% on the basis used here, as compared with the '16% of administered activity,' as he expresses it, and there is thus no substantial disagreement between his work and ours.

It is of interest to calculate the amount of testosterone which must be secreted and metabolized during a 24-hour period to produce the average normal urinary androgen excretion. Such a calculation involves the assumption that the recovery value of 54% (average of 65, 49, and 49%, omitting the hypopituitary *H. S.*) as obtained in hypogonads after testosterone treatment is equal to the recovery from testosterone metabolism in the normal body, and furthermore that the testosterone propionate is or behaves in this respect as the true testis hormone. Taking, however, the average normal daily excretions as 67 I.U., and calculating on the basis that half the weight of the androgens is contributed by each substance, androsterone and dehydroandrosterone, the combined activity equivalents of which represent 7 I.U. per mg., we have a daily urinary loss of 9.6 mg. of urinary androgens. Since, according to our recovery figures this is only 54% of the amount of testosterone metabolized in the body during a 24-hour period the total testosterone used becomes 17.8 mg. per day. This figure must be reduced to the extent that urinary androgens in man have a non-gonadal source. Castrates certainly excrete some androgenic material and Callow (2) has observed as much as 30 to 39 I.U. per day. The proper average value for this allowance must await more extensive studies. The daily dose of testosterone required to replace the activity of the normal testis in man is not known; but from 7.0 to 25 mg. per day of the propionate will develop the secondary sex characters of the hypogonad and cause enlargement of the penis and prostate (8, 14, 15, 16, 17).

SUMMARY

The intramuscular injection of 25 mg. of testosterone propionate per day for periods of 9 to 14 days to 4 eunuchoids brought about rises in urinary androgens from basal levels of 2 to 16 I.U. per day to 44 to 115 I.U. per day and of urinary estrogens from the equivalents of 0.3 to 5 γ of estrone per day to that of 5 to 15 γ of estrone per day. This constitutes a restoration to normal values for both groups of substances. Both androgen and estrogen values fell toward pre-treatment levels when injections stopped. The changes in androgen and estrogen excretion paralleled the physiological effects of testosterone propionate in the organism.

In 3 patients in whom the cause of the hypogonadism was unknown the extent of recovery of administered testosterone was 65, 49, and 49%, on the assumption that half of the excreted material by weight is androsterone and half dehydroandrosterone. In a patient whose pituitary was damaged by a suprasellar cyst, the recovery was 20%.

The rise in urinary estrogens to normal values following androgen administration suggests that the urinary estrogens of normal male urine may be derived in part from the metabolism of the testis hormone. However, the increase in estrogen excretion represents only 0.06% of the androgen injected.

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INFLUENCE OF THE VEHICLE UPON THE LENGTH AND STRENGTH OF THE ACTION OF MALE HORMONE SUBSTANCE, TESTOSTERONE PROPIONATE[1]

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THE CLINICAL usefulness of male hormone (androgen) therapy is handicapped at present by the need of repeated hypodermic injections at short intervals and the expense of the amount of androgenic substance required. Ideally, the aims of substitution therapy with male hormones should be those of giving the smallest amount of material that will, without frequent repetition, maintain an adequate but not excessive level in the tissues and fluids of the body for the longest possible time, that is, providing absorption that will fulfill but not greatly exceed the requirements of the body, and utilizing the least painful and distasteful mode of administration. Thus expediency and inexpensiveness would be combined.

Moreover, such an ideal method of administration would be expected to approach the normal manner of testicular function. The testis contains, and appears to produce, only small amounts of androgenic substances at a time. Yet, in from 1½ to 4½ hours one bull testicle can produce from 8 to 25 international units (I.U.) of androgen, as shown by the in vitro studies of Danby (1) who perfused the testis with bull's blood. In man the urinary excretion alone of androgenic substance is between 40 and 100 I.U. per 24 hours (2, 3, 4). Yet, although studies of castrated and hypogonadal men (5, 6) indicate that the testis is responsible for the major portion of this urinary androgenic activity, there is only a small amount of androgen in the testis at any one time.

If there are large amounts of male hormone in the circulating media of the body, the urinary output of comb growth-promoting substance rises. Increased excretion accompanies elevation of the level of male hormones in the tissues and fluids of the body as shown by studies of persons receiving male hormone therapy (6) and of certain patients with adrenal tumors (7, 8, 9, 5) in whom the production by the body is augmented. As much as 500 I.U. was found in a 24-hour urine sample when a daily dose of 120 mg. of testosterone propionate was taken orally by a 35-year-old man (6). Another patient excreted 264 I.U. in 24 hours when 60 mg. of testosterone propionate

[1] Supported in part by the International Cancer Research Foundation, the Fluid Research Fund of Yale University School of Medicine and the General Education Board of the Rockefeller Foundation.

was ingested daily. Appreciation of the waste involved when the body levels of hormone are high is to be seen not only in the great quantity excreted but also in the fact that clinical benefit was slight. Seemingly, large amounts given orally are too readily absorbed and eliminated. Possible damage may be done to the body by large amounts of hormone, as suggested earlier (10) and recently confirmed (11) with regard to the growth of chicks. The effects of the hormone are widespread, for cardiac enlargement, an influence on the pulse rate and blood pressure, and changes in the muscles, skin (12) and vision have been observed to occur in androgen-treated castrated men (13).

With intramuscular administration of the difficultly-absorbed testosterone propionate, the results are more encouraging. The patient who showed almost no change with ingestion of 120 mg. daily, obtained relief from castration symptoms after receiving daily intramuscular injections of 20 mg. in peanut oil and the urinary excretion of androgens was maintained within the limits determined for normal men. But injections given daily, or for that matter even twice weekly, are troublesome, expensive and induce in some people the feeling of being abnormal. Yet with infrequent injections of the hormone in oil, there is in the blood and tissues either an excessive amount immediately after injection and a low level before the next injection or, if too much material be given, the amount is continuously too large. So with frequent injections the fluctuation of the level of androgens circulating in the body is less but the inconvenience and annoyance to the individual is greater.

Prolonged action of administered material is to be sought. Deanesly and Parkes (14) have clearly shown a means for possibly obtaining such lasting action in the form of intramuscular or subcutaneous implantation of crystalline material which is only slowly absorbed.

In the data presented below we have attempted *a*), comparison of the action of testosterone propionate[2] when subdermally placed in large crystals with that dissolved in various solvents, and *b*), to introduce the androgen while it is dissolved in a solvent or held in a suspension through the skin and then after introduction either cause the vehicle to change to a solid form or the testosterone propionate to be precipitated; the purpose of this procedure was to escape the necessity of incision for implantation of crystalline material.

MATERIAL AND METHODS

The 238 animals used for assay were day-old chicks of inbred stock. Females were used throughout save in parts of experiments 3 and 4. The sex of the birds is easily distinguished at birth in Hallcross[3] chicks by external examination since the males have white down on the top of their heads and the heads of the females are entirely black. This sex marking was found correct in every bird by autopsy at the completion of experiments. These chicks are produced by crossing the Plymouth Rock hen with the Rhode Island Red cock.

[2] Testosterone propionate under the trade name Perandren was furnished through the courtesy of the Ciba Pharmaceutical Company Inc.

[3] Furnished by Hall Brothers of Wallingford, Connecticut.

In experiment 3, male chicks were used for comparison with female chicks. In experiment 4, a comparison was made with the response obtained in male and female White Leghorns.

All injections were made subcutaneously. The same volume of 0.4 cc. of the vehicles was used, since the amount as well as the type of solvent influences the absorption (15) of the hormone. Since the experiments involved introduction of some vehicles at a temperature at which the vehicle was a liquid, the same temperature of from 50 to 55° C. was used for all injections. After injection the site of administration was cooled with a piece of ice. A 13-gauge syringe needle was used as a trocar for implantation of the crystals, which weighed from 3 to 7 mg. apiece.

Vehicles included peanut oil, beef tallow, mutton tallow, spermaceti wax, mineral oil, ethyl alcohol, propylene glycol, palmitic acid, palmitic acid and peanut oil, peanut oil and water, and propylene glycol and water. Two different quantities of testosterone propionate were given, 20 mg. in experiment 1, and 5 mg. in experiments 2, 3 and 4. Injection was given in the form of a single dose on the day after hatching. The birds lived in brooders until they were 70 days of age. Those kept after this age were then transferred to outdoor pens.

The action of the hormone was studied in terms of comb growth. The length, height and width of the comb were measured with a mm. ruler. The sum of these three dimensions was taken for every chick and an average made for each experimental group. Obviously, this sum is not an evaluation of the volume of the comb, for small increases in these figures represent large increases in volume. Due to the irregularities in the shape of the combs it was felt that measurements taken in this manner would be more accurate than those of volume and would be much more simply done. These averages, taken at intervals, are listed in tables 1 to 4.

RESULTS AND DISCUSSION

A comparison was made of the action of subdermal testosterone propionate when in crystal form with that dissolved in various vehicles. Peanut oil, which has been used routinely (16, 17, 18) in intramuscular and subcutaneous injections in human beings, was compared with paraffin oil and with other solvents chosen to provide slow absorption. One approach to this question was the use of a solid as a vehicle; for this, spermaceti and paraffin waxes, mutton and beef tallows were selected because their melting points permitted injection as liquids at temperatures which would not result in burning of the body tissues. Once within the body they were cooled by application of ice to the overlying skin and remained in a solid form.

Experiment 1. With a standard dose of 20 mg. of testosterone propionate, the vehicles are listed in table 1 in their respective order of augmenting both the length and the strength of the action of the dissolved androgen. Least effective were spermaceti wax and mineral oil. Peanut oil, paraffin wax, mutton tallow and alcohol were somewhat better but distinctly inferior to beef tallow. Beef tallow, although a better vehicle than the others, was not as efficient as the direct implantation of crystals (fig. 1). Comb growth at a more rapid rate than that exhibited by control animals lasted for 27 days with the use of beef tallow, for approximately 13 days with mutton tallow and peanut oil, for 9 days with mineral oil, and for 5 days with spermaceti wax. Upon the cessation of androgenic stimulation of comb growth, the cavernous tissue becomes pale and loses its turgidity, and as involution proceeds the color fades to orange and finally to a pale yellow.

TABLE 1

Solvents	Number of birds	Number of days since the single injection was given																	
		5	9[1]	13	17[1]	21	27[1]	36	43	51	58[1]	71	79	86	94	100	113	127	142
Controls	8	10.0	10.7	11.0	14.2	15.9	20.1	23.9	25.6	28.3	32.1	35.2	35.6	35.7	36.8	38.8	38.6	40.5	47.2
Spermaceti wax	7	12.0	13.0	14.3	17.7	17.4	22.2	23.8											
Mineral oil	8	11.1	15.6	17.4	20.2	20.3	24.4	27.9											
Peanut oil	6	11.2	16.3	18.0	21.8	19.8	24.9	27.5											
Paraffin wax	8	10.6	17.5	21.1	26.6	25.6	29.3	27.9											
Mutton tallow	8	12.3	18.3	22.3	25.3	24.1	27.6	28.3	31.9	35.0	36.5	37.7	38.1	39.6	39.8	39.9	39.1	42.6	47.4
Alcohol	3		15.9	23.3	28.9	29.4													
Beef tallow	7	13.7	18.1	25.3	36.0	37.4	43.8	39.8	40.7	42.0	44.6	44.8	44.4	44.4	44.5	44.3	45.6	48.5	50.6
Crystals	8	14.8	23.1	28.7	38.2	49.7	52.3	61.1	65.7	70.7	70.7	75.2	74.5	69.0	59.6	57.8	58.2	55.2	54.7

TABLE 2

Controls	8	10.0	10.8	11.0	14.2	15.9	20.1	23.9	25.6	28.3	32.1	35.2	35.6	35.7	36.8	38.8	38.6	40.5	47.2
Spermaceti wax	8	10.8	13.1	13.7	19.8	20.1	24.6	26.7											
Mineral oil	7	10.3	12.6	14.5	17.9	18.9	22.5	24.2											
Peanut oil	7	11.2	16.3	14.0	19.5	19.0	24.4	26.2											
Paraffin wax	8	11.7	15.6	15.6	20.3	21.4	24.6	27.3											
Mutton tallow	8	11.8	16.0	17.1	21.7	21.1	24.7	26.2	33.6	36.9		39.2	39.6	40.8	40.6				
Beef tallow	8	14.6	20.1	24.2	32.9	34.2	38.4	36.5	33.6	36.9	40.1	39.2	39.6	40.8	40.6	39.9	42.1	44.4	46.4
Crystals	8	13.5	19.3	24.4	34.7	37.2	46.1	39.4	52.1	55.4	57.7	58.1	57.4	56.5	53.4	55.1	52.1	53.4	56.9

Vehicles for testosterone propionate are listed in the order of their augmentation of the length and strength of the androgenic action after a single subcutaneous injection of testosterone propionate. Criteria were the comb growth responses of day-old Hallcross female chicks. Comb growth was measured as the sum in mm. of the length, width and height of the comb. Data in columns marked with ([1]) were obtained by one man, whereas those in unmarked columns were taken by the other author. Table 1 shows the responses to 20 mg. of testosterone propionate, table 2 the responses to 5 mg. of testosterone propionate.

After the 36th day, comb measurements were made only of control birds and of those which had received crystals, beef or mutton tallow. The mutton-tallow group soon adopted a level about that of the controls. The beef-tallow group, after keeping pace for 27 days with the animals receiving crystals, showed a sudden decrease in growth, the comb involuting for a few days followed by a small growth spurt seemingly by hormone produced in the body (fig. 1). Although no great growth ensued, the beef-tallow group retained combs larger than those of controls during the 36th to the 142nd day.

The group receiving crystals exhibited a steep curve of comb growth for 71 days;

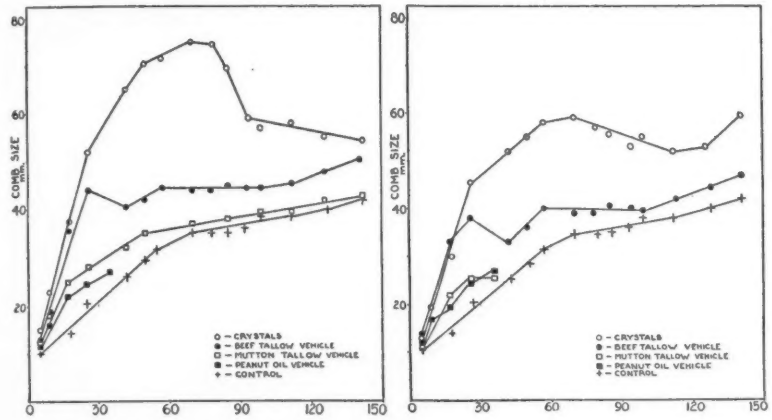

Fig. 1 and 2. THE RATE AND DURATION OF COMB GROWTH IS GREATER WHEN TESTOSTERONE PROPIONATE IS USED IN CRYSTALLINE FORM THAN WHEN DISSOLVED IN VARIOUS VEHICLES (data from tables 1 and 2. The abscissa is graduated in days after a single administration of 5 mg. testosterone propionate). Of the solvents, beef tallow resulted in the strongest and most prolonged action of the hormone. Although the maximum response in comb growth was greater in the birds receiving 20 mg. of testosterone propionate (fig. 1), the same type and duration of response with the various vehicles occurred in birds receiving 5 mg. (fig. 2).

thereafter the involution occurred first with marked and then with lessening rapidity until the comb size approached that of the beef-tallow group.

It is interesting to note that once good development of the comb was attained in the beef-tallow, mutton-tallow, and crystal groups in experiment 1, the size of the comb never decreased to that of control animals, but always remained somewhat larger. Either the hormone-stimulated erectile tissue kept its growth lead in response to body hormones or, less likely, these animals were stimulated to greater hormone production.

Experiment 2. A smaller dose of testosterone propionate, 5 mg., was given in the same vehicles except alcohol as were used in experiment 1. These animals were studied simultaneously with those in experiment 1 so that seasonal variation would not be a factor; the data is referable to the same group of control animals. As shown in table 2 and fig. 2, the magnitude of comb growth was not as great, but the same general types of curves were obtained as in experiment 1. Again spermaceti wax was least effective. The rate of comb growth was no more rapid than in controls after 9 days in the spermaceti wax, after 13 days with mineral oil, after 17 days in peanut oil, paraffin-wax and mutton-tallow groups. Comb growth in birds receiving hormone

in beef tallow kept pace for 27 days with that in birds given crystalline hormone, then showed involution for a few days and subsequent growth to retain over the 142 days of measurement larger combs than those of control birds. Birds receiving crystals exhibited the most rapid rate and greatest extent of comb growth. The combs reached their maximum growth at 71 days, after which time rapid and then slow involution took place.

The rate and magnitude of comb growth in birds receiving crystals and the various solvents showed exactly the same general relationship among responses with different vehicles and crystals whether the dose was 20 mg. or 5 mg. This confirms the findings with each dose.

Significance is attached to the finding that testosterone propionate in beef tallow

TABLE 3

Solvent	Number of birds	Sex	Number of days since the single injection was given			
			15	21	27	38
Controls	5	Female	12.9	18.8	21.7	25.4
Propylene glycol	6	Female	17.8	20.5	23.5	30.2
Propylene glycol and water	5	Female	19.0	21.9	24.4	25.4
Peanut oil and water	5	Female	18.5	22.6	24.4	26.6
Palmitic acid and Propylene glycol	6	Female	19.0	23.0	26.7	27.1
Palmitic acid and Peanut oil	6	Female	19.8	23.0	25.1	29.2
Peanut oil	5	Female	24.6	25.5	29.5	32.0
Beef tallow	5	Female	27.0	30.8	30.8	29.5
Palmitic acid	6	Female	24.5	29.1	32.2	34.6
Crystals	5	Female	36.3	48.0	54.8	55.9
Controls	3	Male	14.7	21.2	27.5	40.5
Propylene glycol and water (hormone in suspension)	3	Male	19.8	27.0	35.2	42.7
Propylene glycol and water	4	Male	19.5	25.0	36.5	
Propylene glycol	3	Male	24.6	30.1	37.1	48.0
Crystals		Male	31.2	42.5	49.8	53.3

Experimental conditions are like those described in tables 1 and 2 save that a group of males is also studied and that propylene glycol and water are added to the solvents in an attempt to precipitate the testosterone propionate in a subcutaneous site. Precipitated testosterone propionate appeared to exert less action than testosterone propionate dissolved in peanut oil. Again, the hard lump crystals were most effective and beef tallow the second best.

gives as good results for 17 days and almost as good for 27 days as those obtained with crystals. Clinical use of beef tallow as a vehicle for testosterone propionate has not been made as yet. The tallow is cheap but there are many other considerations as, for example, uniformity.

Experiment 3. A second approach to the problem of introducing the hormone in a difficultly absorbed form was that of injecting it as a suspension, such as in a mixture of propylene glycol and water. As a variant, the testosterone propionate was injected while completely dissolved in 4 parts of propylene glycol to 1 part of water and by means of a 2-way valve, water was then added through the same inserted needle. The hormone precipitates from propylene glycol when more water is added.

It was further attempted to check whether or not the addition of palmitic acid, which is known to add to the effectiveness of a solution of testosterone (19), would influence the action of testosterone propionate in peanut oil or in propylene glycol.

Tests of the effects obtained with beef tallow, crystals, and peanut oil were repeated for comparison with those found with palmitic acid and with precipitation

of the testosterone propionate. Also, a small series of males was used for comparison with the effects found in females.

The results listed in table 3 show that the precipitation of testosterone propionate subcutaneously does not produce an action equal to that obtained with a peanut oil or a beef tallow vehicle. Testosterone propionate in palmitic acid induced comb growth that was approximately that obtained with beef tallow. It would seem that some fatty acid such as palmitic might possibly be a useful vehicle.

In male chicks, testosterone propionate acts most vigorously in the form of crystals but the rapidity of comb growth in the control males renders less striking the difference between injected and uninjected birds. In the 38 days of measurement there was no proof of a summation of effects from the injected crystals and the body-produced hormone, since the comb growth of males receiving crystals was not as rapid as that of females.

Experiment 4. The growth in White Leghorns was compared with that of the

TABLE 4

Solvent	Number of birds	Sex	Number of days since the single injection was given			
			5	17	21	36
Controls	5	Male	12.6	15.0	18.5	69.0
	3	Female	12.1	15.0	22.8	36.0
Crystals 20 mg.	3	Male	14.6	36.2	57.0	87.0
	2	Female	14.2	32.5	53.0	85.5
Crystals 5 mg.	2	Male	15.0	38.8	56.5	81.3
	1	Female				
Peanut oil 20 mg.	5	Male	14.4	29.4	36.4	79.0
	5	Female	14.8	27.1	32.5	36.7

Day-old White Leghorn male and female chicks were used for comparison with the Hallcross chicks. Rapid growth of the comb occurs much earlier in the White Leghorn control birds.

hybrid bird on which the previous experiments were done. Since the comb of both male and female control White Leghorns grew very rapidly even at early dates, the hybrid used in the previous experiments gave more conclusive data.

It has been reported that crowing can be induced in 10-day-old chicks which received daily injections of 0.5 mg. of testosterone propionate in peanut oil (10). Some of the birds which received 20 mg. of testosterone propionate, crowed at the age of 60 hours. Wing-flapping, strutting and crowing were seen chiefly in animals receiving crystals.

Since Butenandt (20) first obtained crystalline androgenic material in 1931, a series of studies have added much to the effective administration of androgens. Freud (21) stated in 1933 that with greater purification of the extracts of urinary androgens there was less stimulative action on the accessory reproductive organs of the castrated rat. David, Dingemanse, Freud and Laqueur (22) found that the addition of inactive discarded fractions of extracts from urine enhanced the activity of testosterone. In 1936 Miescher, Wettstein and Tschopp (19) pointed out that adding one of various fatty acids increased the effectiveness of a solution of testosterone. Deanesly and

Parkes (23) showed that a larger volume of the oil vehicle augmented the action of testosterone and androsterone.

> It seemed clear, therefore, that the loss of effectiveness on rats of purified urine extracts, and the unexpectedly low effectiveness of crystalline testis hormone in concentrated oil solution, were caused by too rapid absorption from the site of injection, and that absorption could be delayed and the hormone made more effective by the re-addition of discarded fractions or fatty acids, or by increasing the volume of oil used for injection (24).

Then Miescher et al. (25) found that with aliphatic esters the longer the chain of esters the greater the duration of action. They selected testosterone propionate as the compound best suited for duration and intensity of action.

By far the slowest absorption of all is the compressed pellet of dry hormone which therefore cuts down waste and inconvenience. But it must be remembered that human beings by virtue of their greater size, require more hormone than a chick or small rodent. So if pellets are given to human beings, either they must be with surface area and vascularization of surrounding tissue sufficient for absorption of an adequate amount of material or they must be supplemented by other forms of administration. These facts are brought forcefully to mind by the findings that in the monkey a 40 mg. tablet of testosterone propionate will not inhibit a standardized estrogen-withdrawal menstruation, whereas 1 mg. of testosterone propionate daily in 1 cc. of peanut oil will suppress bleeding for months (26). Thus a 40 mg. pellet made to fit a 13-gauge syringe needle did not allow absorption of 1 mg. daily. Similarly, implantation of 40 mg. and 100 mg., respectively, in pellet form in 2 men with low amounts of testicular secretion was not effective, whereas daily intramuscular injections of 10 mg. and 20 mg. in oil, respectively, gave relief from castration symptoms. Further, there are the difficulties of placing large pellets and the possibilities of pellet extrusion and of foreign body reactions.

With the use of long-acting modes of administration there is also the danger of producing a longer effect than necessary. Pellet treatment of certain types of cryptorchidism, which respond to temporary treatment with male hormone (27, 28, 29), might be dangerous. Further, the use of androgens in menorrhagia and metrorrhagia may lead to masculinization (30, 31) or, if the women be in the early stages of pregnancy, possibly to the production of genital abnormalities such as pseudohermaphroditism (32, 33, 34, 35, 31).

SUMMARY

Comparison has been made of the action of testosterone propionate when injected in crystalline form and when dissolved in various solvents. This action was determined in terms of comb growth of day-old chicks following a single injection of the androgen. The following conclusions and suggestions were drawn.

As suggested by Deanesly and Parkes, crystals gave the strongest and longest stimulation, a single injection of 20 mg. lasting for 71 days. Beef tallow was next best, a 20 mg. dose exerting an action like that of crystals for 17 days and continuing to stimulate growth for 27 days. Palmitic acid was also a satisfactory vehicle. Peanut oil, an example of the oils which have

been used routinely in clinical therapy, acted in a lesser manner over a total of 17 days. Spermaceti wax and mineral oil gave lesser effects. Precipitation of the hormone from a solution injected intramuscularly resulted in less comb growth than obtained when the hormone was dissolved in peanut oil. Twenty mg. of testosterone propionate produced a greater duration and maximum of comb growth than 5 mg., but the general type of the response to different solvents was the same with both doses. Pellets of crystalline hormone offer a means of administration which may be perhaps less expensive and more convenient and more practicable clinically than the present means of injecting oil solutions intramuscularly. With implantation methods, the correct doses and the feasibility in human beings have yet to be determined. There are also definite dangers in forms of administration that provide vigorous androgenic stimulation of long duration.

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THE EFFECT OF IODINE ON THE INTERSTITIAL CELLS OF THE TESTIS

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AN UNDERSTANDING of the function of the interstitial cells of the testis has been retarded by the lack of an agent with the capacity of selectively damaging these cells without producing coincident change in the seminiferous tubules. The use of such an agent would answer unequivocally the question of whether the interstitial cells elaborate the androgen, and would be an important aid in the analysis of several problems of the biology of the testis.

That the desired technic had been found was indicated by Bucheim (1) and Lipschütz and Morales (2) when they reported that chronic daily oral administration of iodine, in the form of the tincture mixed with the food, produced regression of the seminal vesicles and testicular damage restricted to the Leydig cells.

Bucheim's experiment utilized a total of 14 animals (rats, mice, and guinea pigs) which received respectively averages of 12, 2.5, and 35 mg. of pure iodine daily for periods varying from 8 days to 4 months. The animals increased in weight under this treatment, but the extent of the growth is not stated. At autopsy the seminal vesicles were found to be greatly reduced in size and almost empty of secretion, and the epithelium in what is described as a state of glandular rest. The testis lesion involved only the Leydig cells which were markedly diminished in size, devoid of droplets, and pyknotic; the tubules were normal provided the dose of iodine had not been too great.

Lipschütz and Morales employed a total of 10 immature rats which received 10 mg. of iodine daily for 71 to 102 days. Their animals showed a considerable retardation of growth under treatment. The emptied seminal vesicles of the treated animals weighed about 35% less than those of controls of similar weight, while the testes were of normal weight and contained spermatozoa. The thyroids were normal microscopically.

Since the number of animals in these studies was small and the results of considerable potential significance, we have attempted to duplicate these effects by a slightly different procedure.

[1] This study was aided by a grant from the Fluid Research Funds of Yale University School of Medicine.

METHOD

Both mice and rats were employed. The mice were of several inbred strains as well as a few hybrids;[2] and at the start of treatment ranged in age from 34 to 238 days. The rats were albino and hooded strains and of 35 to 105 days of age when treatment was started. Instead of adding iodine in the form of the tincture to the food, as did Bucheim and Lipschütz and Morales, the iodine was given subcutaneously in the form of an aqueous solution of C.P. sodium iodide. This method of administration was utilized because of its greater ease and accuracy. The mice received daily 0.1 cc. of a 3.0% solution of sodium iodide (the equivalent of 2.55 mg. pure iodine) for 62 to 131 days. The rats received a daily injection of 0.2 cc. of a 5.9% solution of sodium iodide (the equivalent of 10.0 mg. pure iodine) for 111 to 124 days. Twenty-four mice and 11 rats were treated, while 12 mice and 5 rats served as controls. With both rats and mice the control animals were untreated litter-mates. The treated and control animals were not caged together but, except for the injections, the two groups received identical treatment. All animals were weighed at intervals.

At the end of the period of treatment the animals were killed with ether and autopsied. The testes, seminal vesicles, adrenals, and hypophyses were weighed and sectioned. The seminal vesicles were freed from the coagulating glands and emptied of their secretion before weighing in order to facilitate comparison with the data of Lipschütz and Morales.

RESULTS

At no time was there any depression of body growth. No involution in the adults or retardation of development in the young animals was indicated by the weights of the seminal vesicles which were entirely normal microscopically and characterized by no sign of androgen diminution. Likewise no difference was found in the testis weights in the treated and control animals. The interstitial cells of the testes of the mice were normal in every respect; there was no evidence of damage such as pyknosis, shrinkage, or loss of lipoid as described by Bucheim. In the rat the interstitial cells typically are poorly developed, a fact which makes difficult the detection of damage; however, these cells appeared to be quite normal and were indistinguishable from the interstitial cells of the control rats. The tubules of both rats and mice were entirely normal and spermatogenesis appeared unaffected. In short, no differences could be detected in the genital tracts of the treated and control animals.

In weight and histology the adrenals and hypophyses of the injected animals were quite like those of their controls. No castration changes were seen in the hypophyses. Frequently, especially in the mice, there was some enlargement of the thyroid in the treated animals; but no differences were evident histologically.

COMMENT

The data obtained in this experiment show that iodine administered daily

[2] The mice were obtained through the kindness of Dr. L. C. Strong from his colony.

in the quantity used by Bucheim and by Lipschütz and Morales, but in the form of sodium iodide in aqueous solution given subcutaneously, produces no demonstrable damage in the interstitial cells of the testis, and results in no change in the weight or histology of the seminal vesicles. Ostensibly, the same effect should be produced by orally administered tincture of iodine and subcutaneously administered sodium iodide since it has been shown (3) that free iodine cannot exist in tissue fluids but is rapidly converted into iodide ion and organic iodides. Our iodine dose is, therefore, undoubtedly as great as in the cited work and is definitely absorbed. Indeed, the amount of iodine entering the blood is probably greater in our animals in view of the possibility of the passage unabsorbed of some iodine through the digestive tract in the orally treated animals.

The difference in the effect of iodine on the reproductive tract in our animals and the animals of Bucheim and of Lipschütz and Morales is very probably explicable by the difference in the mode of administration. The well known gastric and intestinal disorders of iodism would probably follow chronic oral ingestion of even small quantities of iodine, and would lead to partial anorexia and reduced food consumption. At any rate, growth was retarded in at least one of the cited experiments and the condition of the reproductive system as found by Bucheim and by Lipschütz and Morales is very similar to that seen in partial inanition (4).

In view of this fact and of our own observations we are of the opinion that small quantities of iodine over a long period do not directly produce selective damage to the interstitial cells of the testis and coincident diminution of androgen production as indicated by atrophy of the seminal vesicles.

SUMMARY

In an attempt to duplicate the reported finding that chronic daily oral administration of iodine, as the tincture, produces testicular damage affecting only the interstitial cells and diminution of androgen production, mice and rats were given subcutaneous injections of sodium iodide daily over periods ranging from 62 to 131 days. No differences between the treated animals and their untreated litter-mate controls were observed in the weight and histology of the testes, seminal vesicles, hypophyses, and adrenals, nor in the rate of growth.

It is suggested that the reported positive effects were the result of partial inanition which was the immediate effect of orally administered iodine.

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THE EFFECTS OF OVARIECTOMY ON THE ADRENAL GLANDS OF THE ALBINO RAT

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THAT AN adrenal-ovary relationship exists is established, but its nature and significance are as yet incompletely known. This experiment was carried out in order to study morphologic changes in the adrenal glands following ovariectomy, with the hope of elucidating further the connection between these structures. Of the vast amount of literature which deals directly and indirectly with the subject, reference will be made only to some of the more important and pertinent reports in the discussion of results.

MATERIALS[1]

A study was made of 99 pairs of adrenal glands obtained from female albino rats of the Wistar strain, treated as follows: weaning to 3-month group, 22 control and 21 test animals, operated on during the 4th week of age and autopsied at 3 months; weaning to 6-month group, 25 control and 25 test animals, operated on during the 4th week of age and killed at 6 months; and 3- to 6-month group, 4 control and 3 test animals, operated on at 3 months and killed at 6. Details of the procedure of ovariectomy, of the control operation, of the care of the rats, and of the method of autopsy may be obtained from the report of Freudenberger and Billeter (1), on the weaning to 6-month group.

METHODS

The adrenal glands were studied as to weight of whole gland, cortex and medulla; weight of water, lipins and defatted remainder; volume of whole gland, cortex, cortical zones and medulla; and microscopic anatomy. To make these observations each gland of a pair was weighed separately. One member of each pair was fixed in Regaud's fluid or Bensley's modification of Helly's fluid. The other, in a majority of cases, was dried and defatted, but in a few was fixed in 10% neutral formalin, sectioned with the freezing microtome, and stained with Herxheimer's solution of scarlet red. The methods used in making the studies and the statistical procedures employed in analyzing metric data will be considered next.

Method of Determining Weight of Cortex and Medulla

For this purpose the paper outline method was used, following the procedure outlined by the author (2). As has been mentioned by others, the left adrenal gland

[1] The experiments were conducted by Drs. C. B. Freudenberger, O. A. Billeter and P. M. Howard, who have studied other changes in these rats; I wish to thank them for the material.

of the albino rat is nearly always heavier than the right, a weight difference of 5% or more having been noted in several series. In attempts to apply exact methods such differences cannot be neglected. The question arose whether or not the consideration of only one gland of a pair would give correct results, that is, would give results similar to those obtained by studying both glands. To settle the question with regard to estimating the weight of the cortex and medulla by the paper outline method, 10 pairs of adrenal glands from normal female albino rats were used. A standard was made by determining cortex and medulla weight for each gland of a pair and combining them. That method of study of one gland which gave results most closely approximating the values determined from both glands of a pair could be considered best. It was found best to use, alternately, heavier and lighter glands of successive pairs, applying per cent values derived from the study of one gland to the total weight of the pair. Incidentally, the heavier glands of this series weighed 5.2% more than the lighter, the difference being due largely to the cortex, which weighed 5.2% more. The medulla of the heavier glands weighed but 0.3% more.

It may be stated here that in connection with the determination of water, lipin and remainder content of the adrenals a similar problem arose, whether or not the use of one adrenal would do as well as the use of both of a pair. The study of 10 pairs of adrenals made possible the conclusion that one gland of each pair could be used and that no definite order of selection was necessary. The information obtained by these trials was applied in the conduct of the experiment. The data, omitted to conserve space, are on file at the Department of Anatomy, University of Utah.

Method of Determining Volume of the Adrenal Gland and its Component Parts

In using the paper outline method to determine the weight of the cortex and the medulla, the volume of the whole adrenal and these parts may be obtained thus: weight of paper outline drawings × thickness of gland represented by each drawing ÷ weight of paper per unit area × magnification squared. The weight per unit area of the paper used, 'Artesian Ledger,' was determined by weighing 10 sample pieces, each 100 square centimeters in area, at 10 different times during the year. The volume of the zones of the cortex cannot be derived by this method, for toward either end of a series of sections, as the zones are appearing or disappearing, they cannot be clearly distinguished at magnifications suitable for outlining a whole section. A method of obtaining approximate values was developed.

The adrenal gland was assumed to be a sphere, and each of the cortical zones and the medulla, concentrically contained spheres. The radius of a sphere equal to the volume of the adrenal, already determined by the paper outline method, was calculated. The average thickness of each zone was determined by making 20 measurements of each zone in different parts of 5 centrally located sections. To determine the approximate volume of the zona glomerulosa, the average thickness of that zone was subtracted from the radius of the sphere equal in volume to the whole adrenal. The resultant value was treated as the radius of a concentrically contained sphere, its volume calculated, and subtracted from the volume of the whole adrenal. This furnished an approximation of the volume of the zona glomerulosa. The volumes of the other zones were determined in a similar manner. The procedure may be summed up in the following formulas.

V_a = volume of whole adrenal, determined by paper outline method.
V = volume of sphere of same volume as V_a.
r = radius of sphere of volume V.
r − thickness of zona glomerulosa = r_1.

V_1 = volume of sphere with radius of r_1.
$V - V_1$ = volume of zona glomerulosa, V_g.
r_1 — thickness of zona fasciculata = r_2.
V_2 = volume of sphere with radius of r_2.
$V_1 - V_2$ = volume of zona fasciculata, V_f.

The volume of the zona reticularis was calculated by proceeding along the same line. Volumes were calculated in cubic millimeters.

Method of Determining Weight of Water, Lipins and Defatted Remainder

Following removal from the rat at autopsy each adrenal of a pair was weighed separately. The gland to be studied as to water and lipin content was then placed in an open vial in an oven maintained at 56° C., and dried to constant weight. The loss in weight represented the water content. The dried gland was then placed in a modified form of Soxhlet extractor and treated for periods of 24 hours with benzol, absolute alcohol, chloroform and acetone. The gland was again dried to constant weight, the decrease being construed as due to lipins removed by the solvents used. What was left of the gland is referred to as remainder. It may be noted here that all measurements obtained were subjected to biometric analysis. For groups of less than 15 measurements Fisher's (3), small sample method was used, or a correction factor was introduced into the formulas used for larger samples.

Methods Employed in the Microscopic Examination of the Adrenal Glands

In making general observations the intensity of staining, tinctorial differences, vascularity, distinctness of separation between zona glomerulosa and zona fasciculata, arrangement of cell columns of the zona reticularis, and the cortico-medullary boundary were studied. Intensity and the color of the staining reaction were compared only where similarly fixed control and test specimens had been stained together. It became apparent, early in the course of these studies, that it was as important to study a large number of specimens in making microscopic observations as in dealing with weights or volumes. In an attempt to learn the cause of weight and volume changes observed, cell regeneration, cell destruction and cell size were studied.

Regeneration of cortical cells is generally believed to take place in the outer part of the cortex. Zwemer (4), and others maintain that the capsule cells become transformed into cortical cells, but since counts of these could not be made no further consideration was given to this process. In several control and test specimens nuclei apparently undergoing amitotic division were seen. Careful study showed these nuclei did not belong to cells lining the sinusoids, but since the possibility of distortion could not be eliminated, such nuclei were not counted. Mitoses could be recognized with certainty, so a quantitative study of their occurrence was undertaken as an index of the rate of cell regeneration. Five centrally located sections of each gland studied were examined completely for mitoses at a magnification of 1000 diameters. The sections thus examined were outlined on paper and their volume determined. It was then possible to estimate the number of mitoses per cubic millimeter of cortical tissue, and, having already determined the volume of the whole cortex, the number of mitoses per whole cortex.

As expressed recently by Hoerr (5), and Zwemer (4), it is widely held that a structural and functional gradient exists in the adrenal cortex. New cells are formed in the outer part of the cortex, move or are moved into the zona fasciculata where they function, and finally reach the zona reticularis where they are destroyed. An attempt at a quantitative study of the rate of cell destruction in the zona reticularis

broke down because it was not possible to make a sharp distinction between degenerating and non-degenerating cells.

Cell size was determined by an indirect method in the zona fasciculata. It was not studied in other zones because of the marked variation in cell size, the indefiniteness of cell boundaries, and the marked differences in vascularity. A square delimited by hairs which marked off an area of known size was placed in the eyepiece of the microscope, and the number of nuclei per field counted. Ten fields in each of 5 sections of a gland, a total of 50 fields per gland, were studied. Knowing the size of the field and the thickness of each section, it was possible to calculate the number of nuclei per unit volume of zona fasciculata and per whole zona fasciculata. Dividing zone volume by number of nuclei gave an estimate of cell volume. This procedure overlooked space occupied by the sinusoids, but was employed because of its value in furnishing comparative rather than absolute data. As a check on the reliability of this method for determining cell size, comparison may be drawn with the values obtained by Jackson, (6). He employed a paper outline method, assumed the cells to be spheres, and found that in albino rats more than 10 weeks old the average diameter of the cells in the outer part of the zona fasciculata was from 14 to 15μ. In the study here reported the average diameter of the fasciculate cells of control rats 3 months old was 14μ.

RESULTS

Weaning to Three-Month Group

Body weight and body length. Mean values and other statistical data are recorded in table 1. The details of these changes and growth curves have been reported by Freudenberger and Howard (7). The spayed rats were 22.3% heavier and 3.3% longer than their control mates, both differences being statistically significant. In some manner removal of the ovaries affected development, resulting in longer and heavier rats.

Weight of whole adrenal, cortex and medulla. As recorded in table 1, it was found that the mean weight of the adrenals of the spayed rats was 8.3% less than that of the controls. The significance ratio of 2.8 was interpreted as indicating a difference probably significant. The adrenal cortex of the spayed rats was 9.5% lighter, the medulla, 8.3% heavier than those of the controls. The cortex difference was statistically significant, that of the medulla possibly so. These findings indicated that the smaller adrenal of the spayed rat was due either to a reduction in size or a failure of normal development of the cortex. The next step was aimed to determine what had happened to the cortex.

Volume of whole gland, cortex, medulla and cortical zones. The total volume of the test glands was 15.1% less than that of the controls and the difference was significant (table 2). Further analysis showed the cortex was responsible for this difference, since in the test rats it was 16.4% smaller, whereas the medulla was 1.8% larger. The cortical difference was significant, the medullary difference was not. The volumetric studies, therefore, were in agreement with the ponderal. Volumetrically, the zona glomerulosa and zona fasciculata of the adrenals of the spayed rats were decreased 5.8 and 22.6% respectively, but the zona reticularis was 1.3% larger. Only the decrease in size of the zona fasciculata was significant, and it alone was responsible for more than 96% of the decrease in volume of the cortex and of the whole gland.

TABLE 1. COMPARISONS OF BODY WEIGHT, BODY LENGTH, WHOLE ADRENAL WEIGHT, CORTEX WEIGHT AND MEDULLA WEIGHT

	No.	Controls Mean and S.D.	No.	Spayed Mean and S.D.	Difference and P.E.	S.R.	% diff.
Weaning to 3-month group							
Bd. wt. gm.	22	169.0±10.9	21	206.7±19.3	37.7±3.3	11.3	22.3
Length cm.	22	18.80±0.37	21	19.41±0.51	0.61±0.10	6.5	3.3
Adrenal mg.	22	41.55±5.42	21	38.09±6.09	−3.46±1.22	2.8	− 8.3
Cortex mg.	22	38.82±5.16	21	35.13±5.84	−3.69±1.16	3.2	− 9.5
Medulla mg.	22	2.73±0.42	21	2.96±0.54	0.23±0.10	2.2	8.3
Weaning to 6-month group							
Bd. wt. gm.	25	212.7±18.2	25	249.3±36.5	36.6±5.6	6.5	17.2
Length cm.	25	20.40±0.58	25	20.50±0.64	0.10±0.12	0.9	0.5
Adrenal mg.	16	53.49±9.21	15	35.61±5.69	−17.88±1.90	9.4	−33.4
Cortex mg.	16	49.80±8.77	15	31.98±5.22	−17.82±1.79	9.9	−35.8
Medulla mg.	16	3.68±0.66	15	3.64±0.61	−0.04±0.16	0.3	−1.3
Three to 6-month group							
Bd. wt. gm.	4	199.0	3	234.5	35.5		17.8
Length cm.	4	19.90	3	20.40	0.50		2.5
Adrenal mg.	4	45.83	3	30.07	−15.76		−34.4
Cortex mg.	4	42.42	3	26.72	−15.70		−37.0
Medulla mg.	4	3.40	3	3.34	−0.06		−1.7

S.D.=Standard deviation. P.E.=Probable error. S.R.=Significance Ratio. Significance ratio is an expression of the probability that differences found are due to the error of random sampling. When the ratio is 3.0 or more the chances are less than 1 in 20 that the difference is accidental. Interpretation of the individual S.R. values may be made from Pearl, (8) Table A, Appendix III, page 438.

TABLE 2. COMPARISONS OF VOLUME OF WHOLE ADRENAL, CORTEX, CORTICAL ZONES AND MEDULLA

Volume cu. mm.	Controls Mean and S.D.	Spayed Mean and S.D.	Difference and P.E.	S.R.	% diff.
Weaning to 3-month group: 22 controls and 21 spayed					
Whole adrenal	10.0569±2.0783	8.5339±1.6498	−1.5230±0.3851	4.0	−15.1
Cortex	9.3702±1.9638	7.8345±1.5555	−1.5357±0.3634	4.2	−16.4
Z. glomer.	1.2449±0.1995	1.1724±0.1764	−0.0725±0.0387	1.9	− 5.8
Z. fascic.	6.5691±1.7048	5.0860±1.1631	−1.4831±0.2990	5.0	−22.6
Z. retic.	1.5561±0.4511	1.5761±0.4657	0.0200±0.0943	0.2	1.3
Medulla	0.6867±0.2773	0.6994±0.3061	0.0127±0.0600	0.2	1.8
Weaning to 6-month group: 16 controls and 15 spayed					
Whole adrenal	12.8099±3.0756	9.4835±1.5926	−3.3264±0.5880	5.7	−26.0
Cortex	11.5902±2.9413	8.2629±1.4854	−3.3273±0.5594	5.9	−28.7
Z. glomer.	1.4859±0.2753	1.3156±0.2439	−0.1703±0.0632	2.7	−11.5
Z. fascic.	7.4448±1.9677	5.3314±1.0041	−2.1134±0.3751	5.6	−28.4
Z. retic.	2.6596±0.9126	1.6158±0.3068	−1.0438±0.1628	6.4	−39.2
Medulla	1.2196±0.4920	1.2206±0.2775	0.0010±0.0959	0.01	0.1
Three to 6-month group: 4 controls and 3 spayed					
Whole adrenal	9.1036	6.8519	−2.2517		−24.7
Cortex	8.4190	6.0626	−2.3564		−28.0
Z. glomer.	1.2332	0.9974	−0.2358		−19.1
Z. fascic.	5.3544	3.8681	−1.4863		−27.8
Z. retic.	1.8316	1.1972	−0.6344		−34.6
Medulla	0.6846	0.7893	0.1047		15.3

Water, lipin and remainder content. Sixteen control and 16 test glands were employed. The data obtained are recorded in table 3. In total weight the test glands were 8.7% lighter than the control, thus being representative of the larger groups from which they were selected at random. Water and remainder were reduced about as much as total weight, 7.1 and 9.6% respectively, but the lipins had decreased in weight by 15.7%. This dis-

TABLE 3. COMPARISONS OF WEIGHT OF WHOLE ADRENAL, WATER, LIPINS AND REMAINDER

Weight mg.	Controls Mean and S.D.	Spayed Mean and S.D.	Difference and P.E.	S.R.	% diff.
Weaning to 3-month group: 16 controls and 16 spayed					
Whole adrenal	40.83±5.72	37.28±5.59	−3.55±1.39	2.5	− 8.7
Water	28.28±4.44	26.26±4.01	−2.02±1.01	1.9	− 7.1
Lipins	5.36±0.81	4.52±0.78	−0.84±0.20	4.3	−15.7
Remainder	7.19±0.86	6.49±0.99	−0.70±0.23	3.0	− 9.6
Weaning to 6-month group: 9 controls and 9 spayed[1]					
Whole adrenal	46.12±5.71	30.38±2.67	−15.74±1.50	10.5	−34.1
Water	33.20±3.76	21.82±1.92	−11.38±1.01	11.3	−34.3
Lipins	5.21±1.35	3.58±0.45	− 1.63±0.34	4.8	−31.3
Remainder	7.71±0.92	4.98±0.47	− 2.73±0.25	11.1	−35.4
Three to 6-month group: 3 controls and 3 spayed					
Whole adrenal	45.40	30.07	−15.33		−33.8
Water	30.91	20.59	−10.32		−33.4
Lipins	6.07	3.76	−2.31		−38.0
Remainder	8.42	5.71	−2.71		−32.1

[1] Corrections introduced in calculations for small size of sample.

proportionate reduction of the lipin material in the adrenals of the ovariectomized rats is in agreement with the morphologic observations of Andersen and Kennedy (9), who observed a decrease in adrenal cortical lipoids of spayed rats.

Microscopic findings. The glands studied had been prepared for microscopic examination as follows: a majority were fixed in Bensley's modification of Helly's fluid or Regaud's fluid and stained either with Harris' hematoxylin and eosin Y or Heidenhain's iron hematoxylin in pairs consisting of a test and a control; a few were fixed in 10% neutral formalin, sectioned with the freezing microtome, and stained with Herxheimer's solution of scarlet red. Of 18 pairs of control and test glands stained together, 7 test glands stained more intensely, 9 the same, and 2 less intensely than the controls. The color of the cortical cell nuclei ranged from pale blue to deep purple; pale blue nuclei were apparently more numerous in test glands, but no counts were made. An approximation of the vascularity of the glands was made by rating them from 1 to 4. For 22 control glands the average was 2.9; for 22 test glands, 2.5. If any difference existed at all, the control glands were slightly more vascular than the test. There were no differences in the general appearance of the zona glomerulosa and zona fasciculata, except that in sec-

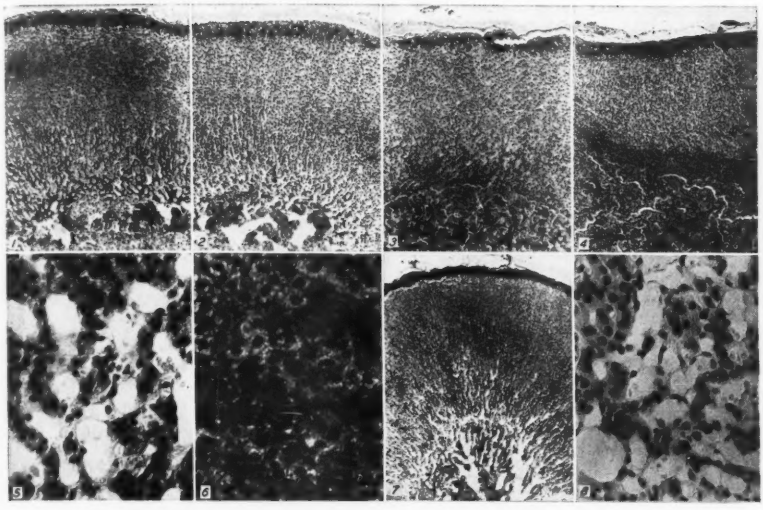

Fig. 1. PORTION OF CENTRAL SECTION THROUGH ADRENAL OF WEANING TO 3-MONTH CONTROL. Note the sharp separation of z. glomerulosa from z. fasciculata, the distention by blood of sinusoids in the reticular zone, the thickness of the columns of reticularis cells; and a group of large pale cells, between medulla and reticular zone, which should be compared with cells of the fasciculate zone. ×40.

Fig. 2. PORTION OF CENTRAL SECTION THROUGH ADRENAL OF WEANING TO 3-MONTH TEST. Note in addition to the features mentioned for the preceding figure, the thinning of the cell columns of the z. reticularis. ×40.

Fig. 3. PORTION OF CENTRAL SECTION THROUGH ADRENAL OF WEANING TO 6-MONTH SUMMER TEST. Compare it with fig. 4, 7. Note the less distinct separation between glomerulosa and fasciculata, the compact arrangement of the reticularis, and the absence of signs of destruction of the reticularis cells. ×40.

Fig. 4. PORTION OF CENTRAL SECTION THROUGH ADRENAL OF WEANING TO 6-MONTH WINTER TEST. Note the thinner cortex, the degree of distinctness between glomerulosa and fasciculata, the compact arrangement of the reticular zone (compare with fig. 7), and the tangential dark band at the junction of fasciculate and reticular zones. ×40.

Fig. 5. PORTION OF THE RETICULAR ZONE OF FIG. 2 AT HIGHER MAGNIFICATION. Note evidences of cell destruction, including thinned cell columns, empty strands of sinusoidal reticulum, and pyknotic nuclei. The few pigmented cells present can be distinguished only by their greater granularity. ×190.

Fig. 6. PORTION OF THE DARK BAND IN FIG. 4, LOCATED BETWEEN FASCICULATE AND RETICULAR ZONES. Note the crowded tangentially elongated nuclei which belong mainly to sinusoidal reticulum cells. Pyknotic nuclei are numerous, as are the more refractile and granular pigmented cells. ×190.

Fig. 7. PORTION THROUGH THE CENTRAL SECTION OF THE ADRENAL OF A WEANING TO 6-MONTH WINTER TEST. The z. glomerulosa is distinctly demarcated from the z. fasciculata, which was not the case in a majority of the other test glands of this group. The sinusoids of the reticular zone are distended. In the outer part of the reticular zone there has been marked loss of parenchymal cells. ×40.

Fig. 8. HIGHLY MAGNIFIED PORTION OF THE RETICULAR ZONE OF FIG. 7. Note the decrease in number of reticularis cells, the empty strands of sinusoidal reticulum, the pyknotic nuclei, and the granular pigmented cells.

tions stained with scarlet red the amount of lipoid droplets was greatly reduced in the test glands. Comparison of the degree of distinctness of separation of the glomerular from the fasciculate zone in 22 pairs of control and test glands revealed the separation was sharper in 17 controls, the same in 4 pairs, and sharper in 1 test. Thus, the zona glomerulosa was less clearly demarcated from the fasciculata in spayed rats (fig. 1, 3). The zona reticularis showed marked variations in the thickness of cell columns and the compactness of their arrangement, which occurred as often in the test as in the control glands and probably represented different functional states (fig. 1, 2, 3,

4). In the reticular zone, particularly next to the medulla, groups of cells much larger and paler staining than the rest of the reticularis cells were noted in both control and test glands (fig. 1, 2). They resembled closely the cells in the inner part of the fasciculata, and in many but not all instances, could be traced through a series of sections to a connection with the fasciculata. It appeared that the reticularis was not a complete layer between the fasciculata and the medulla, but that it was interspersed with and broken

TABLE 4. COMPARISONS OF NUMBER OF MITOSES AND OF NUMBER OF NUCLEI

Value compared	Controls Mean	Spayed Mean	Difference and S.E.	t	P
Weaning to 3-month group: 10 controls and 10 spayed					
Mitoses per cu. mm. of cortex	49.9	74.0	24.1±26.2	0.920	.4–.3
Mitoses in whole cortex	477.7	538.3	60.6±174.0	0.343	.8–.7
Weaning to 6-month group: 10 controls and 10 spayed					
Mitoses per cu. mm. of cortex	20.0	30.0	10.0±14.2	0.707	.5–.4
Mitoses in whole cortex	206.7	266.2	59.5±129.2	0.460	.7–.6
Weaning to 3-month group: 15 controls and 15 spayed					
Nuclei per .001 cu. mm. z. fasc.	651.0	776.8	125.8±30.6	4.110	less than .01
Nuclei in whole z. fasc., in thousands	4128.2	3764.5	−363.7±358.0	1.016	.4–.3
Weaning to 6-month group: 12 controls and 10 spayed					
Nuclei per .001 cu. mm. z. fasc.	624.0	725.5	101.5±36.1	2.812	.02–.01
Nuclei in whole z. fasc., in thousands	4175.4	3652.1	−523.3±319.7	1.637	.2–.1

S.E.=Standard Error. t=a ratio calculated by dividing the difference between means by the S.E. of the difference. P=an expression of the probability that a difference is due to the error of random sampling. The value of P is dependent upon t and the number of samples compared. Values of P may be found in Fisher, (3), Table 4. The lower the value P is, the less likely is a difference accidental. A value of P of .05 or less is considered to indicate a significant difference because the chances are 1 in 20 or less that the difference is accidental.

up by prolongations of the fasciculata which often extended to the medulla. From a comparison of 19 pairs of glands it was concluded that these masses of cells were larger and more abundant in 11 control glands, the same in 7 pairs, and more abundant in 1 test gland. If these cell masses are portions of the fasciculata their diminished amount in the test glands concurs with the previously mentioned finding of a reduced fasciculate zone. Examination of the cortico-medullary boundary revealed no differences between tests and controls. In general, cortical cells rested directly on medullary ones or were separated by a single layer of stroma cells. For reasons given in the discussion of methods, mitotic activity was determined quantitatively as an index of the rate of new cell formation. The data obtained are recorded in table 4. It was found that mitoses were most numerous in the outer part of the zona fasciculata, next most frequent in the glomerulosa, and very rare in the reticularis. Both per cubic millimeter of cortex and per whole cortex mi-

toses were more numerous in the test than in the control glands, but neither of the differences was statistically significant. As evidenced by mitosis cell regeneration was as rapid, if not more, in the test as in the control glands. Cell regeneration, it was concluded, could probably be dismissed as a factor in producing the decrease in size of the adrenals following ovariectomy. As explained in the section on methods, cell size was determined indirectly by counting the nuclei in fields of known area and thickness. The data, presented in table 4, shows that in test glands the number of nuclei per 0.001 cu. mm. of zona fasciculata was significantly greater than in control glands, but that there was no significant difference between mean values for the number of nuclei per whole zona fasciculata. This indicated a significant reduction in size but not in number of cells of the zona fasciculata. Calculation of cell volume from these data showed that in test glands as compared with control the fasciculate cells were 14% smaller. Although the observations made in this experiment did not indicate its extent, some of the reduction in cell volume must have been due to the disproportionate decrease in lipin content. If it be recalled that the zona fasciculata of test glands was 22.6% smaller in volume than that of the control glands, it is seen that the reduction in cell volume of 14% accounted for approximately two-thirds of the diminution in volume of the zone. Two characteristic features were found in the reticular zone of test glands. Evidences of cell destruction, consisting of marked cytoplasmic vacuolization, or condensation and deep eosinophilic staining of the cytoplasm, pyknosis and karyorrhexis, all were more marked in the test than in the control glands (fig. 2, 5). These changes were encountered most frequently in the outer part of the reticular zone. The second observation concerned the pigmented cells which have long been described as a normal constituent of the zona reticularis. A few pigmented cells were observed in the control glands, many more in the test glands; and more frequently, partly pigmented cells were seen, these patches being larger and more numerous in the test-gland cells. The pigmented cells and patches were brownish-yellow in color, and consisted of a diffuse coloration plus rather fine granules of irregular shape. Vacuoles of various sizes also occurred in the pigmented cells, and appeared to be more numerous and larger in the test gland cells. The pigmentation was not related to fixation, for it was the same in glands fixed with formaldehyde, modified Helly's or Regaud's; nor was it affected by staining with iron hematoxylin or hematoxylin eosin. In frozen sections stained with scarlet red, the pigmented cells and patches stained a diffuse pink, the vacuoles a deeper pink, and the granules a deep, translucent red. But few of the nuclei of these cells appeared to be degenerating; most of them resembled the nuclei of the cells which lined the sinusoids. Probably they were reticulo-endothelial cells engorged with cellular debris.

Summary. Rats spayed after weaning and killed 2 months later were found to be heavier and longer than controls of the same age. The adrenal glands of the spayed rats were smaller, due chiefly to a significant decrease in the size of the zona fasciculata. The factors apparently responsible for the diminution in size of the zona fasciculata were decrease in cell size and exces-

sive destruction of cells; rate of cell regeneration, as evidenced by mitotic rate, was as great as or greater than that observed in the control glands. In addition, the test glands seemed to stain more intensely, had a less clearly demarcated zona glomerulosa, fewer and smaller masses of large pale cells in the reticular zone, and more and larger partially and completely pigmented cells (probably phagocytes) in the reticularis. There was a disproportionately large reduction of lipin material in the test glands.

Weaning to Six-month Group

Body weight and body length. The details of these changes have already been reported by Freudenberger and Billeter (1). It was found, as recorded in table 1, that the spayed rats were 17.2% heavier and 0.5% longer than the controls. Only the weight difference was significant. From observations at the time of autopsy it appeared that much of the greater weight of the ovariectomized rats was due to extensive amounts of fat in the form of subcutaneous and retroperitoneal accumulations.

Weight of whole adrenal, cortex and medulla. As shown in table 1, the adrenals of the spayed rats were 33.4% lighter than those of the controls. This significant difference was the result of a marked reduction in the size of the cortex, which was 35.8% lighter in the spayed rats than in the controls. The difference in weight of the medulla was only 1.3%, and not significant.

Volume of the whole adrenal, cortex, medulla and cortical zones. The data, given in table 2, show the test glands were significantly reduced in volume. The whole gland was 26.0% smaller, the cortex being responsible, for it was 28.7% smaller. The difference in medullary volume was only 0.1% and not significant. All the cortical zones of the test glands exhibited a decrease in volume, that of the glomerulosa being 11.5% and probably significant, fasciculata 28.4% and significant and reticularis 39.2% and significant. In absolute terms the zona fasciculata exhibited the most marked reduction, but from the relative viewpoint the reticularis had undergone the greatest decrease.

Water, lipin and remainder content. The observations made (table 3) showed that total adrenal weight of the tests compared with the controls was reduced 34.1%, water content 34.3%, lipins 31.3% and remainder 35.4%. Unlike the weaning to 3-month group, in which lipins were reduced about twice as much as water and remainder, all three components of the glands of the 6-month group were reduced significantly and to about the same extent.

Microscopic findings. The control and test glands compared were not stained together, so observations concerning intensity of staining and color of nuclei were not made. The vascularity of the glands of both groups appeared the same except for a distinctly more vascular zone in the outer part of the reticularis of the test glands. This zone, the vascularity of which approached hemorrhagic proportions in some of the glands, was present in 14 of 17 test specimens and in none of the controls. The 3 test glands in which

it was not present were from rats which experienced their main growth during the summer; the others, from a group which grew during the winter. As already pointed out by Freudenberger and Billeter (1), the changes in the winter group were more marked than in the summer animals. This has been the case regarding other adrenal observations, such as weight and volume changes, the values being closer to control ones for the summer tests. (Compare fig. 3 with fig. 4 and fig. 7.) In test glands the zona glomerulosa was less clearly demarcated from the zona fasciculata (fig. 1, 3, 4). The reticularis exhibited striking differences visible at low magnification. Besides the greater vascularity, already mentioned, in and about this zone numerous large pigmented cells were seen (fig. 6, 8). The area, in many instances, seemed compressed or collapsed, the nuclei and cell bodies of both parenchymal and stromal cells having a tangential instead of the usual radial disposition (fig. 4, 6). Cell destruction was intense (fig. 7, 8). The pigmented cells, many of which reached a size two or three times that of the largest fasciculate cells, were much more numerous and larger in the test than in the control glands, and showed more and larger vacuoles. The large cell masses in the reticular zone, interpreted as prolongations of the fasciculata, were larger and more numerous in the control glands. The cortico-medullary border was of similar structure in tests and controls, and did not differ from that described for the younger group. The data for mitotic activity (table 4) indicate that although mitoses were more numerous in the test glands both per cubic millimeter of and per whole cortex, the differences were not statistically significant. The number of nuclei per cubic millimeter of and per whole zona fasciculata (table 4) indicate that the fasciculate cells were significantly smaller in volume but not in total number. The reduction in estimated cell volume amounted to 14%, but the reduction in volume of the zona fasciculata was 28.4%. As in the weaning to 3-month group, diminution in cell size accounted for only part of the general decrease.

Summary. In general, the changes observed were qualitatively similar to those observed in the weaning to 3-months group. The great reduction in size of the test adrenals was due to a diminution in volume of all three cortical zones. Water, lipins and remainder were reduced about as much as the weight of the whole gland. Rate of formation of new cells by mitosis was the same as or greater in tests than in controls. Reduction in volume of fasciculate cells accounted for about half the decrease in volume of the zona fasciculata. Cell destruction, particularly in the outer part of the reticularis, was far more intense in the test glands, and probably was responsible for the remainder of the decrease in volume of the zona fasciculata and much of the marked decrease of the reticularis. In the test glands the zona glomerulosa was less clearly demarcated from the zona fasciculata than in the control glands. The test adrenals showed fewer and smaller clumps of large pale cells in the reticularis than did the control glands. A layer or zone, located in the outer part of the reticularis, characterized by vascular engorgement, intense cell destruction, compression or collapse of parenchymal and stromal cells, and numerous pigmented cells was found in every winter test but in

none of the summer tests or controls. The pigmented cells, probably phagocytes, were larger and more numerous in the test glands.

Three to Six-month Group

Because only 4 control and 3 test animals were available, the results will not be cited at length. Like the weaning to 6-month group, the test rats were significantly heavier but not longer than the controls (table 1). The adrenals were 34.4% lighter in the tests, due to a 37.0% reduction in the weight of the cortex (table 1). Volume changes (table 2) were similar to those found in the weaning to 6-month group except that the zona glomerulosa of the test glands was reduced to a greater extent. Water, lipin and remainder content of test glands were each reduced about as much as the whole gland was. Microscopically the test glands of this group resembled closely those of the weaning to 6-month group, the main difference being the absence of a highly vascular zone in the outer part of the reticularis. Thus, the adrenals of the rats ovariectomized at 3 months of age reached, in 3 months, a state essentially similar to that attained in 5 months by the adrenals of rats spayed shortly after weaning. It would seem that the changes induced in the adrenal by ovariectomy were not dependent on chronologic time primarily, but that the physiologic age of rat during the period of ovariectomy was the determining factor.

DISCUSSION

Brief mention will be made first of the results obtained by others. Not including the reports of Freudenberger and Billeter (1), and Freudenberger and Howard (7), with which material this paper deals, 24 separate papers have been found in the available literature dealing with the effects of ovariectomy on the adrenal glands. A study of these revealed great diversity in animals used, age of animals when spayed, duration of the experiment, season of the year, and treatment of the adrenals. Much of the apparent confusion of results disappears if the reports are classed according to the animals used. Of those who used rabbits, Ranieri (10), Renon and Delille (11), Schenk (12), Marrassini and Luciani (13), Kolde (14), and Friedl (15), found an hypertrophy, due, where such studies were made, to an increase in size of the cortex, particularly the zona fasciculata and zona reticularis. Soli (16), found an increase in size of the adrenals for 1 month after ovariectomy, then, a decrease; Livingston (17), observed no change comparing the entire test with the entire control group, but an hypertrophy if litter mates were compared; Dick and Curtis (18), found no change. In rabbits most workers have found the adrenals larger following ovariectomy. Using guinea pigs, Marrassini and Luciani (13), Cirillo and Guardavacarro, (19), and Leinati (20), found the adrenals increased in weight following spaying; Zalesky (21), found no definite size change; Soli (16), noted first an increase, then, a decrease; Moore (22), who conducted his experiment for a much longer time than the others, observed a definite decrease in size. As in rabbits, the most common finding in guinea pigs has been an increase in size. Masui and Tamura, (23), Howard-Miller, (24), Deansley (25), and Poll (26), ovariectomized mice; none of them recorded weight changes and none found microscopic changes. In ovariectomized dogs Theodosieff (27), found an hypertrophy due to enlargement of the glomerulosa and fasciculata; Pianese (28), observed no changes. In spayed chickens Soli (16), notedlatrophy of the

adrenals after a long period of time. Zalesky (29), working with ground squirrels, found intensive destruction of the reticularis and replacement by a fibrous ring around the medulla, but recorded no weight changes. It is impossible to make comparisons with these results because of the great variations in the factors mentioned above, and because of the apparent difference of reaction of different animals. Considering the reports of those who have worked on rats we find an almost unanimous opinion. Hatai (30), found the adrenals decreased in weight in all the ovariectomized groups he used. Andersen and Kennedy (9), reported changes varying according to the time of spaying and the duration of the experiment. Rats spayed at 21 days of age and killed 1 to 7 weeks later showed no size change of the adrenals, though those of the latter group had a decreased amount of lipoid in the fasciculate zone. Rats spayed after sexual maturity and killed one week later had hypertrophied adrenals with marked engorgement of the inner third of the cortex; killed from 3 to 8 weeks later, all had atrophied adrenals except one group of 4 rats killed in autumn, the adrenals of which were similar in size to the control glands. Microscopically the atrophied adrenals showed a narrowed cortex, a decreased amount of lipoid in the fasciculate zone, and, in the inner half of the cortex, congestion, narrowed cell columns, decreased cell size, many pyknotic nuclei, and deeper eosinophilic staining of the cytoplasm. Ellison and Burch (31), found that the adrenals of the adult female rats, spayed for 25 days, were decreased in size. Winter and Emery (32), using sexually mature but not full-grown rats, spayed for 3 weeks or more, found a decrease in weight of the adrenals that was statistically significant. With the exception, therefore, of certain groups used by Anderson and Kennedy, those who have used rats have found a decrease in size of the adrenals following removal of the ovaries.

In practically every detail comparable observations made in the work here reported agree with those of preceding workers. It may be concluded, because of the unanimity of results, that, except for experiments of very short duration, removal of the ovaries of albino rats causes the adrenals to become smaller than normal in size because of the decrease in cortex.

Any interpretation of the morphologic reflections of functional alterations must be based on some anatomic concept of cortical activity. The most widely accepted idea, more recently expressed by Hoerr (5), and Zwemer (4), as well as by many others, holds that an anatomic as well as a functional gradient exists in the cortex. New cells, it is believed, are formed in the zona glomerulosa, move or are moved inward toward the medulla, develop and function in the fasciculate zone, and finally are destroyed in the reticularis. If this concept is correct, then increased rate of cell destruction in the zona reticularis may be due either to toxic substances in the blood or to increased rate of use of cortical cells. That toxic substances were present in the blood of the spayed rats has not been ruled out, although aside from the decreased metabolic rate and general activity, the ovariectomized rats appeared to be in good health. The morphologic alterations observed were suggestive of an increased rate of function with incomplete ability to respond to it as an indirect result of removal of the ovaries. Cell regeneration, evidenced by mitosis, was as rapid in the test adrenals as in the control, but the fasciculate cells were smaller in size and were being destroyed more rapidly than in the control glands. An increased functional demand on the cortex, causing hur-

ried passage of cells from the outer to the inner zones, could both explain and receive support from the findings in the test glands of a less distinct separation between glomerular and fasciculate zones, decreased size of fasciculate cells, and increased destruction in the reticular zone. Such an explanation would assume that an increased burden had devolved upon the adrenals. In partial support of this suggestion, two recent reports may be mentioned. Thorn and Harrop, (33), showed that certain sex hormone preparations, like adrenal cortical hormone, have 'some degree of "sodium retaining effect".' Moon (34), presented strong evidence that the adrenal cortex forms a female sex hormone. Perhaps ovariectomy places upon the adrenals an excessive demand for one or more of these functions.

Others postulate a fourth cortical zone, a subdivision of the reticular, directly related to the gonads. While for some animals like the mouse there is excellent evidence for distinguishing such a zone none that is conclusive has yet been presented for the rat. Were it true that a certain portion of the cortex was directly related to the ovary, the rapid destruction of cells in the outer part of the reticular zone might be interpreted as due to lack of ovarian secretion. But the failure of this phenomenon to occur in summer rats, observed both by Andersen and Kennedy (9), and the author, is against such an idea, for ovarian secretion was lacking in both summer and winter rats.

Ellison and Burch (31), concluded that ovariectomy interfered with hypophyseal secretion of adrenotropic hormone because estrin repaired the atrophy of the adrenals resulting from long-term castration in spayed but not in spayed-hypophysectomized rats. This assumption, in its entirety, seems unwarranted, for hypophysectomy alone causes an atrophy of the adrenals which well may be of a different nature than that following ovariectomy, so could not be expected to be repaired by estrin. Cutuly (35), observed a 73% reduction in size of the cortex of the adrenals of adult female rats 30 days after hypophysectomy, a shrinkage far more abrupt and extensive than occurred in spayed rats. Furthermore, Emery and Winter (36) showed that spaying did not affect materially the adrenotropic potency of the pituitaries of donors; and Winter and Emery (32), demonstrated that ovariectomy did not alter the ability of one adrenal to undergo compensatory hypertrophy when the other was removed. Although the possibility remains that estrin repairs the adrenal atrophy of ovariectomy by acting through the hypophysis, it has by no means been proved.

CONCLUSIONS

From a study of 10 pairs of adrenal glands it was concluded that in determining the weight of the cortex and medulla by the paper outline method, the use, alternately, of heavier and lighter glands of successive pairs gave results most closely approximating values obtained by using both glands of a pair. In determining water, lipin and remainder content the use of either gland of a pair gave results practically the same as those derived from the use of both glands.

The mean adrenal weight of 21 test rats, spayed shortly after weaning and killed at 3 months of age, was 8.3% less than that of 22 control rats. This was due to a significant difference in the size of the cortex, amounting to 9.5%. In the weaning to 6-month group the mean adrenal weight of 15 tests was 33.4% less than that of 15 controls, due to a significant difference of 35.8% in the size of the cortex. In the younger group the diminution in size was due almost entirely to the zona fasciculata; in the older group all three zones were smaller.

As an indication of the rate of new cell formation, the incidence of mitoses in the cortex was studied in 10 test and 10 control animals in each group. In both the mitotic rate was the same in tests as in controls. The size of the fasciculata cells was determined by an indirect method in 15 tests and 15 controls of the younger group, and in 10 tests and 12 controls of the older group. In both groups the test fasciculata cells were 14% smaller in volume than the control cells. These differences were significant, and explained in part the reduction in volume of the fasciculate zone. Destruction of cells, especially in the outer part of the reticular zone, was more intense in the tests than in the controls of both groups, much more so in the older group.

The most constant microscopic finding was an increase in the number, size and degree of vacuolization of pigmented cells in the zona reticularis. The increase was greater in the 6-month than in the 3-month tests. The pigmented cells possibly are phagocytes, increased in size and number because of increased destruction of cells.

The reduction of water and remainder content in the adrenals of the 3-month tests was proportionate to the whole adrenal weight reduction, but lipin material was reduced almost twice as much. In the 6-month tests the reduction in weight of water, lipins and remainder was in the same proportion as total weight reduction.

Three rats, spayed at 3 months of age and killed at 6, compared with 4 controls, showed adrenal changes similar to those of the weaning to 6-month tests. Evidently physiologic time was more important than chronologic time in producing the adrenal changes.

The following interpretation of the findings is suggested: removal of the ovaries leads to an increased functional demand on the adrenal glands, as evidenced by increased rate of cell destruction. The heightened function shortens the life cycle of the cortex cell, which then does not have an opportunity to reach full size. New cell formation by mitosis does not keep pace with cell destruction. As a result the cortex decreases in size.

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CLINICAL AND LABORATORY NOTES

REFRACTORINESS FROM PITUITARY THYROTROPIC EXTRACTS*

WINDSOR C. CUTTING, G. BERNARD ROBSON
AND
KENDALL EMERSON, JR.†

ALTHOUGH THE exact nature of refractoriness to anterior pituitary preparations has not been explained, extracts have been prepared which have potent thyrotropic activity but which cause only occasional refractoriness. It is presumed that in these preparations the foreign substances responsible for the refractory state have been eliminated. Werner[1,2] in 1936 and 1938, reported continuing activity in the majority of guinea pigs after 80 days of injection with an extract made by the flavianate method of Evans, Meyer and Simpson,[3] as modified by Meyer. In attempting to repeat this work, evidence concerning the effect of age of the pituitary extracts on refractoriness was obtained.

Method. Anterior lobe extracts of the pituitary‡ were made by the flavianate method of Meyer as used by Werner and injected daily in amounts of from 5 to 50 mg. into young virgin guinea pigs. B.M.R. determinations were made weekly, using an apparatus similar to that described by Tainter and Rytand.[4] At the termination of an experiment, the thyroid glands of the guinea pig were sectioned and examined histologically. Throughout the experiments the guinea pigs were fed either the low iodine diet described by Friedgood,[5] or the diet used by Werner, which consisted of alfalfa hay, lettuce, oats and carrots.

Results. In the first group of experiments, acetone dried beef pituitary powder was used for the preparation of the extracts. This was obtained from two different sources, the material from each source having been prepared over two years before the time the extract was made, although in one case, at least, it had been kept for this entire time at about 0° C. Separate groups of guinea pigs were fed Werner's diet and Friedgood's diet as described above with no difference in the results. Since there was a considerable mortality from hyperthyroidism during the early weeks, only guinea pigs are considered which lived at least 30 days.

Out of 33 guinea pigs which lived for 30 days after the injections were begun, all but 2 had become refractory as judged by metabolic rate determinations, and at

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‡ We are indebted to Sharpe and Dohme, of Baltimore, and to Mr. Robert Fox of Greenwald Inc., Baltimore, for supplies of pituitary powder and fresh pituitaries.

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40 days, 1 of these 2 had also become refractory. At from 14 to 21 days, however, they had all had at least a 50% increase in O_2 consumption. The thyroid glands of these guinea pigs showed varying degrees of activity on histological examination. Those from guinea pigs whose metabolic rates had been back to normal for some time were generally not hyperplastic. Those from guinea pigs which had just become refractory showed slight evidence of activity, and those which died while the O_2 was elevated showed extreme hyperplasia of the glands. In general, the correlation between B.M.R. and degree of thyroid activity was quite close, although the B.M.R. appeared to fall to normal slightly before the histological appearance had returned entirely to normal.

In the second group of experiments, the acetone dried powder was made at the beginning of each experiment from fresh glands and used at once, or extract was made from fresh glands without drying in acetone. Separate groups of guinea pigs were fed Werner's diet and Friedgood's diet as described above with no difference in the results.

Of 15 guinea pigs alive at 30 days, 11 had metabolic rates increased by 20 to 80%, and 4 had normal B.M.R. At 50 days, 4 had metabolic rates increased from 25 to 80%, and 11 were normal. The histological appearance again correlated almost perfectly with the level of O_2. The greatest response in metabolic rate was comparable to that obtained in the first series.

In summary, refractoriness occurred in 94% of 33 animals given extract from old pituitary powder for 30 days, and in only 26% of 15 animals given extract from fresh glands for 30 days, although at 50 days refractoriness was present in 74% of the latter group.

Discussion. It is thus evident that while extract made from old acetone dried anterior pituitary is of the same potency as that made from fresh glands for the production of hyperthyroidism in guinea pigs, refractoriness occurs much earlier from it than from the latter preparation.

However, even with the latter preparation, the percentage of refractoriness increases with time. It is rather difficult to understand why somewhat less success in preventing refractoriness was had than that reported by Werner with a similarly prepared extract, although it must be noted that some of his guinea pigs were refractory at 80 days when he terminated the experiment. Since diet was demonstrated not to explain the difference, it may be supposed that slight differences in the extracts, such, for instance, as less frequent preparation of new batches, or differences in the type of guinea pig, are responsible for the discrepancy.

By using the flavianate method of preparation of the thyrotropic fraction of the anterior pituitary it is possible to greatly delay refractoriness, which suggests that refractoriness is due to the body's reaction to foreign substances, presumably contaminating tissue proteins, which are largely eliminated by this method of preparation. Dried pituitary which has been stored for long periods apparently undergoes changes which make it impossible to remove so completely the substances which induce refractoriness.

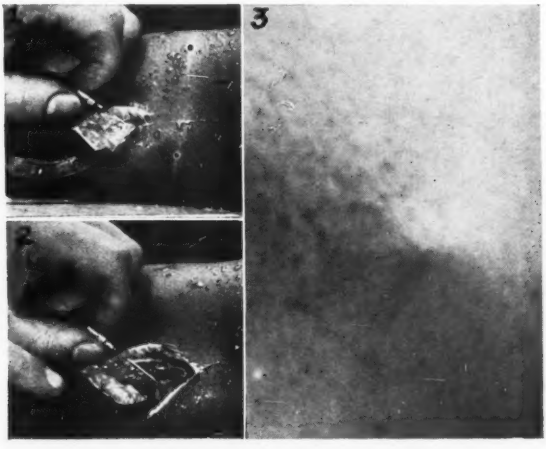
Summary. Refractoriness to thyrotropic hormone is prevented or greatly delayed by using the flavianate method of preparation and fresh pituitary glands. Similarly made extract from glands which had been stored for long periods induced early refractoriness, although the potency for causing immediate thyroid hyperplasia was unchanged. The type of diet had no effect on the appearance of refractoriness.

LOCAL LIPOID ATROPHY AND LIPOID DYSTROPHY

ADVANTAGES OF LIMITING SITES OF INJECTION

BARNETT ALPERT AND EDGAR A. FERGUSON*

OVER THE PAST 7 years, routine physical examinations have been made on 430 insulin-treated diabetic patients in the out-patient department of the hospital. There was lipoid atrophy at the site of injection of standard insulin in 32 or 7.4% of these patients. In 20% of these, atrophy was present in 2 or more places. Eight of them were 30 years of age, 11 under 50 and 13 over 50 years of age. Twenty of these patients were under observation from 4 months to 5 years, 10 from 5 to 15 years and 1 for 17 years before atrophy was observed. In many instances patients were not



cognizant of the atrophy until they came to the clinic. The very thin individuals noticed the atrophy when they began to gain weight.

It is apparent that since only 7% of the group developed fat atrophy, these constitute a group of individuals who are predisposed to such changes. The inoculations were given either directly into the subcutaneous fat or as close to the muscle layer as possible. The risk of muscle irritation and muscle atrophy suggests that caution be exercised in the selection of the length of needle used.

There was no evidence of muscle involvement in our group. The ½-inch needle was used for injection and thus the risk of muscular trauma was avoided. Figure 1 illustrates a dissection of the site of injection with ½-inch needle and figure 2 shows the site of injection with a 1-inch needle.

Figure 3 illustrates the arm of a white male of 50 years of age. He has been a

* From the Metabolic Laboratory and Diabetic Service of Henry M. Feinblatt of the Kings County Hospital, Brooklyn, New York.

known diabetic for 5 years and was treated intermittently during this time. He is subject to insulin shock when using standard insulin. Protamine insulin in a dose of 10 U produces painful local swelling which persists from 6 to 10 weeks. The illustration shows a reaction which is 2 weeks old. The patient is now taking another modified insulin which is most satisfactory.†[1]

In the case of patient *14* in the table of case reports, the atrophy of the inner aspect of the right thigh had been present for 3 years and followed intermittent injec-

TABLE 1. CASE REPORTS

Patient	Age Yr.	Sex	Years before appearance	Location of Atrophy
1	55	M	7	dorsum of arms; lateral aspect of thighs
2	60	F	1	dorsum of arms
3	17	M	3	lateral aspect of left thigh
4	31	M	8	lateral aspect of right thigh
5	25	M	5	outer aspect of left thign
6	36	M	1 & 8 mo.	lateral aspect of right thigh
7	40	F	13	outer aspect of right thigh
8	28	F	3	anterior aspect of thighs
9	14	F	4	outer aspect of thighs
10	17	M	11	dorsum of arms; outer aspect of thighs
*11	50	M	2	outer aspect of thighs
12	21	F	12	outer aspect of thighs
13	15	F	1	outer aspect of thighs
14	23	M	9	dorsum of right arm; outer, inner and front of right thigh
15	64	F	10	dorsum of right arm
16	44	F	3	dorsum of arms
17	50	F	2	dorsum of right arm
18	61	F	4 mo.	dorsum of right arm
19	45	F	2	outer aspect of thighs
20	53	F	3	dorsum of arms
21	48	F	1	dorsum of arms; outer aspect of thighs
22	41	F	8 mo.	dorsum of right arm
23	53	F	14	outer aspect of thighs
24	57	F	2	dorsum of arms
25	42	M	5	dorsum of left arm; outer aspect of thighs
26	42	F	11	dorsum of right arm
27	64	F	13	dorsum of arms
28	68	F	1	dorsum of arms
29	52	M	22	anterior aspect of left thigh
30	60	F	15	lateral and anterior aspects of right thigh
31	36	F	5	lateral, inner and front aspects of thighs; left forearm
32	40	F	4	inner and front aspect of thighs; left forearm

* FEINBLATT, H. M.: *M. Record* 145: 366. 1937.

tion of standard insulin for 2 years. Before this time, the arm was used. The left thigh was injected continuously for more than 2 years. No other site of injection was used during this time. While there is slight swelling of the outer aspects, no atrophy is evident.

Site of injection. Lipoid dystrophy has been observed at the site of injection of standard insulin, protamine insulin and crystalline insulin. In a selected group, the same site of injection was used continuously for 2 years. During this time the dystrophy has persisted but the area has not increased in size.

Clinicians had advised that the site of injection of insulin be changed frequently.

† Hexamine insulin, prepared in Metabolic Laboratory, Kings County Hospital, Brooklyn, New York.

[1] FEINBLATT, H. M.: J. Lab. & Clin. Med. 24: 337. 1939.

TABLE 2. LOCAL DYSTROPHY CASES REPORTED IN LITERATURE

Reference	Age Yr.	Sex	Years before appearance	Location of Atrophy
1	49	F	8 mo.	dorsum of arms; outer aspect of thighs
1	48	F	14 mo.	outer aspect of right thigh; front of left thigh; left lower quadrant of abdomen
2	26	F	4	outer aspect of thighs
3	39	F	12 mo.	dorsum of left arm
3	8	M	2	dorsum of arms
4	56	F	1	dorsum of arms
5	37	F	14 mo.	outer aspect of thighs
6	54	F	8 mo.	dorsum of left arm; outer aspect of left thigh
7	35	M		dorsum of left arm; left forearm; front of left thigh
8		F	3 mo.	dorsum of arms; outer aspect of thighs; back; breasts
9	50	F	7 mo.	outer and anterior aspects of thighs
9	45	F	9 mo.	outer aspect of thighs
9	53	F	1	anterior and outer aspects of thighs
9	54	F	8 mo.	front and outer aspects of thighs
9	38	M	2	left buttock
10	45	F	7 mo.	dorsum of arms; lower right abdomen
10	20	F	6 mo.	buttocks; anterior thighs

[1] McCORMIC, V. O., AND T. G. MOORHEAD: *Irish J. M. Sci.* p. 727. 1929.
[2] RABINOWICH, I. M.: *Canad. M. A. J.* 21: 67. 1929.
[3] CARMICHAEL, E. A., AND G. GRAHM: *Lancet* 1: 601. 1928.
[4] AVERY, H.: *Brit. M. J.* 2: 597. 1929.
[5] DAVISON, R.: *Calif. & Western Med.* 26: 210. 1927.
[6] MENTZNER, S. H., AND E. S. DUBRAY: *Calif. & Western Med.* 26: 212. 1927.
[7] LAWRENCE, R. D. *Lancet* 1: 1328. 1928.
[8] DEPISH, L. R.: *Wien. med. Wchnschr.* 80: 168. 1930.
[9] DEPISH, F.: *Klin. Wchnschr.* p. 1965. 1926.
[10] BOLLER, R.: *Klin. Wchnschr.* 9: 2433. 1930.

It is questionable whether such a change is desirable. It appears that where multiple sites of injection are used, there is more tendency toward multiple areas of lipoid atrophy. Inasmuch as the areas of dystrophy are insensitive to pain, and the absorption is as adequate as in any other areas, there appears to be no reason why the same site of injection of insulin should not be used over a period of years.

Summary. Out of 430 insulin-treated diabetic patients 32 or 7.4% developed local lipoid atrophy. During the period of dystrophy, if the inoculations are continued, the dystrophy continues and the development of atrophy is delayed. It appears more desirable to use the local site, which has become insensitive to pain, than to use multiple sites for inoculations.

PERSISTENCE OF ESTROGENS IN THE BLOOD AFTER THE MENOPAUSE

EVAN SHUTE*

WALDEYER in 1870 gave definite form to the belief that the ovaries ceased to function at the menopause. This conclusion has not been questioned until quite recently. Frank,[1] Fluhmann[2] and others[3] have lately found evidence of the presence in the blood and urine of estrogens even long after the cessation of the menses. The source of this estrogen has not been established. It may be suspected, of course, that it has much the same origin as it had before this epoch, viz. the ovaries, although it is known that the anterior pituitary[4] and the adrenal cortex[5] also produce estrogens. Some few instances of conception after the termination of menstruation at the climacteric are well authenticated, and in such women, of course, there must have been good ovarian function. Very recently Wallart and Scheidegger[6] have reported a careful histological study of post-menopausal ovaries these showed a high degree of similarity to those of earlier life. However, their findings are in contrast to those of other investigators.[7,8]

Two years ago we observed that some patients occasionally showed considerable amounts of estrogen in the blood even long after the cessation of the menses. In following up this idea we treated a small group of cases of senile vagino-vulvitis[9] with anti-estrogens with striking success. Since that time we have carried out qualitative blood estrogen determinations[10] on many of our post-menopausal private patients. Most of these women could not be asked to submit to repeated tests in order to determine whether or not an estrogen cycle was still in existence. Our data, therefore, can have only suggestive value and we hope others with better opportunities will be able to carry out this study in a more conclusive manner.

In this 2-year period the author has done blood estrogen tests on 82 such women, who ranged in age from 25 to 86 years, and were, on the average, 53 years old. It should be stated that 21 of these patients were seen for conditions which could not be called 'menopausal' by any means. These women had ceased to menstruate for from 2 months to 27 years, on the average for 7 years. Sixty-eight of these women were tested but once and 46 of these tests showed a high level of blood estrogen. Twelve were tested 2 to 4 times within a single 28-day period of time, and of these, 9 showed a high estrogen once. In short, 55 or 67% of the 82 women showed the presence of a considerable blood estrogen on one or more tests, and only 3 women

* From the Department Obstetrics and Gynecology, University of Western Ontario, London, Canada.

[1] FRANK, R. T.: *New York State J. M.* 34: 1009. 1934. Abst. *Surg., Gynec., & Obst.* 63: 7. July 1936.

[2] FLUHMANN, C. F.: *Am. J. Obst. & Gynec.* 32: 612. 1936.

[3] TAYLOR, H. C., AND R. MILLEN: *Am. J. Obst. & Gynec.* 36: 22. 1938.

[4] PFALTZ, H.: Festschrift f. E. C. Barrell, Basel, 1936.

[5] ENGLEHART, E.: *Zentralbl. f. Gynäk.* 61: 1098. 1937.

[6] WALLART, J., AND S. SCHEIDEGGER: *Arch. f. Gynäk.* 165: 188. 1937. Abst. *J. Obst. & Gynec., Br. Emp.* 45: 563. 1938.

[7] ESCH, P.: Abst. in *Zentralbl. f. Gynäk.* 60: 1863. 1936.

[8] NOVAK, E., AND E. YUI: *Am. J. Obst. & Gynec.* 32: 674. 1936.

[9] SHUTE, E. V.: J. A. M. A. 110: 889. 1938.

[10] SHUTE, E. V.: *Am. J. Obst. & Gynec.* 35: 970. 1938.

adequately tested for cycles did not show them. There was no difference found in the groups showing 'menopausal' symptoms and those who came for quite different complaints—an observation which has puzzled us greatly. Admittedly this data is extremely imperfect, but it does indicate that the high output of prolan A found in the urine after the cessation of cyclic menses[11,12] may be associated with at least an occasional high level of estrogen in the blood stream. The most characteristic feature of the climacteric is probably the gradual development of a state of the ovaries in which they are refractory toward anterior pituitary gonadotropic hormone[13] but the degree of their response or lack of response must vary within wide limits and may not be adequately shown by histological study of the ovaries.

This observation of the persistence of estrogen cycles in the majority of post-menopausal women of any age, if substantiated, should have great significance in respect to the therapy of post-menopausal pathological states. It seems to be accepted very generally that the logical agents to use in such treatment are estrogens, and all dosages have been recommended, from the minute to the enormous. The minute doses may be effective largely by mental suggestion, as Pratt[14] has indicated so convincingly. The huge doses must produce inhibition of the anterior pituitary's gonadotropic function, and so can scarcely be regarded as 'replacement therapy'—they must really be anti-estrogenic in their final effect.[15] The author has had success with the almost exclusive use of anti-estrogens in the management of post-menopausal complaints, acting on an assumption for which he finds little evidence, viz. that ovarian cycles should cease at the climacteric. It seems, indeed that the whole basis of the hormonal treatment of post-menopausal patients needs to be studied further, without regard to a priori conceptions of the rapid and general cessation of ovarian function after the termination of cyclical menstruation. One cannot but be a little skeptical of the high percentage of cures of a great variety of menopausal states produced by the administration of estrogenic agents in widely differing dosage, as reported by so many authors. At the very least, therapy of these patients should be preceded by some attempt at analysis of their hormonal constitution.

My thanks are due Dr. G. K. Wharton for his permission to use data from many of his post-menopausal patients.

Summary. Evidence is presented for the persistence of considerable amounts of estrogens in the blood in the majority of women even long after the termination of cyclical menstruation at the menopause.

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[11] Englehart, E., and E. Tschernz: *Zentralbl. f. Gynäk.* 60: 790. 1936.
[12] Albright, F.: *Endocrinology* 20: 24. 1936.
[13] Watson, B. P., P. E. Smith and R. Kurzrok: *Am. J. Obst. & Gynec.* 36: 562. 1938.
[14] Pratt, V. J., and W. L. Thomas: *J. A. M. A.* 109: 1875. 1937.
[15] Israel, S. L., and C. Mazer: *J. A. M. A.* 36: 445. 1938.

ASSOCIATION NOTICE

SCIENTIFIC PROGRAM OF THE TWENTY-THIRD ANNUAL MEETING

New Jefferson Hotel, St. Louis, Missouri

FRIDAY, MAY 12

9:30 A.M.

Changes in Plasma and Red Blood Cell Sodium, Potassium and Water on Low Sodium, High Potassium Diets in Normal Humans, in Asthenia and in Adrenal Insufficiency. MARY PETERMAN, *Madison, Wisconsin.*

Clinical and Chemical Observations on Adrenalectomized Dogs Maintained by a Diet High in Sodium Salts and Low in Potassium. R. A. CLEGHORN, C. W. J. ARMSTRONG AND D. C. AUSTEN, *Toronto, Canada.*

Cortin and the Sodium Factor. FRANK A. HARTMAN, *Columbus, Ohio.*

The Use of Radioactive Sodium and Potassium in Studies on Adrenocortical Physiology. EVELYN ANDERSON, MICHAEL JOSEPH AND HERBERT M. EVANS, *Berkeley, California.*

The Use of Desoxy-Corticosterone Acetate in the Treatment of Adrenal Insufficiency. KENDALL EMERSON, JR., GEORGE W. THORN AND R. PALMER HOWARD, *Baltimore, Maryland.*

Intersexuality in the Adult Brown Leghorn Male as a Result of Estrogenic Treatment During Early Embryonic Life. L. V. DOMM, *Chicago, Illinois.*

Studies in the Metabolic Effects of Testosterone Propionate in Eunuchoidism and in Normal Men and Women. ALLAN T. KENYON, KATHRYN KNOWLTON, IRENE SANDIFORD, F. C. KOCH AND GERTRUDE LOTWIN, *Chicago, Illinois.*

Chemical Studies Concerning the Inactive Androgens of Human Male Urine. D. ROY MCCULLAGH, *Cleveland, Ohio.*

Production of Genital Growth in the Male After the Age of Puberty. WILLARD O. THOMPSON AND NORRIS J. HECKEL, *Chicago, Illinois.*

2:00 P.M.

Quantitative Studies on the Hormones of Human Pituitaries. EMIL WITSCHI AND G. M. RILEY, *Iowa City, Iowa.*

Results of Further Studies on Anterior Pituitary Extracts. J. B. COLLIP, A. H. NEUFELD AND O. F. DENSTEDT, *Montreal, Canada.*

On Effects of Anterior Pituitary Hormones on Carbohydrate and Fat Metabolism. OSCAR RIDDLE AND TELLEF SENUM, *Cold Spring Harbor, Long Island, N. Y.*

The Effect of Anterior Pituitary and Adrenal Cortex on Carbohydrate and Protein Metabolism. C. N. H. LONG, *New Haven, Connecticut.*

Studies on the Anterior Pituitary Factor which Depresses the R. Q. of the Glucose-Fed Rat. J. D. GREAVES, H. E. JOHNS AND I. K. FRIEBERG, *St. Louis, Missouri.*

Ketone Body Formation in the Liver. MARY SCOTT WELCH AND CART F. CORI, *St. Louis, Missouri.*

Progonadotropic Sera of Animals Treated with Hypophyseal Extracts. PHILIP A. KATZMAN, NELSON J. WADE AND EDWARD A. DOISY, *St. Louis, Missouri.*

The Inhibition of the Action of Human Pituitary Gonadotropic Hormone by a Specific Antihormone. K. W. THOMPSON, *New Haven, Connecticut.*

The Histopathology of the Pituitary of the White Rat Injected with Follicular Hormone. ARTHUR WEIL AND BERNHARD ZONDEK, *Chicago, Illinois and Jerusalem, Palestine.*

The Effect of Sex Hormones on the Anterior Hypophysis of Thyroidectomized Rats. WARREN O. NELSON, *Detroit, Michigan.*

7:00 P.M.

ANNUAL DINNER

President's Address. DAVID P. BARR, Busch Professor of Medicine, Washington University School of Medicine, *St. Louis, Missouri.*

Address: Diabetes Mellitus in the Light of our Present Knowledge of Metabolism. C. N. H. LONG, Sterling Professor of Physiological Chemistry, Yale University School of Medicine, *New Haven, Connecticut.*

SATURDAY, MAY 13

9:00 A.M.

Some Effects of Continued Injections of Stilboestrol in Rats. J. A. MORRELL, *New Brunswick, New Jersey.*

The Mechanism of Estrin Therapy in the Relief of Dysmenorrhoea. SOMERS A. STURGIS AND FULLER ALBRIGHT, *Boston, Massachusetts.*

Time Differences in Onset of Uterine Bleeding after Withdrawal of Estrogen and of Progestin in Rhesus Monkeys. PHILIP E. SMITH AND EARL T. ENGLE, *New York, New York.*

Histological Changes in the Senile Vagina Induced by Estrogenic Therapy Administered Orally and by Inunction. RITA S. FINKLER AND WILLIAM ANTOPOL, *Newark, New Jersey.*

Studies of the Metabolism of Androgens in Women. E. C. HAMBLEN, R. A. ROSS, W. KENNETH CUYLER, MARGARET BAPTIST AND CATHERINE ASHLEY, *Durham, North Carolina.*

Survival, Structure and Function of Pituitary Grafts in Untreated Rats and in Rats Injected with Estrogen. DORIS PHELPS, E. T. ELLISON AND JOHN C. BURCH, *Nashville, Tennessee.*

Heat Labile Factors in the Diet of Cats Necessary to Prevent Gonadal Deficiencies. F. M. POTTENGER, JR. AND D. G. SIMONSEN, *Monrovia, California.*

A Comparative Study of the Effects of Male and Female Sex Hormones on the Human Vaginal Smear. N. O. ROTHERMICH, *Columbus, Ohio.*

The Assay of Gonadotropic Substances. JOHN S. EVANS, LEONARD R. HINES, ROGER F. VARNEY, JOSEPH J. CEITHAML AND F. C. KOCH, *Chicago, Illinois.*

The Assay of Progesterone by the Production of an Artificial Pregnancy Response of the Feline Uterus. H. B. VAN DYKE AND JOSEPH S. CHEN, *Peiping, China* and *New Brunswick, New Jersey.*

The Effect of Certain Sex Hormones on the Rabbit's Resistance to Infection with Vaccinia. DOUGLAS H. SPRUNT, *Durham, North Carolina.*

2:00 P.M.

Studies of Vigor in Senescent Rats. R. G. Hoskins, Helen M. Levene and Sylvia Bevin, *Boston, Massachusetts.*

The Endocrine Control of Blood Serum Amylase Activity. The Effect of Alteration of Function of the Pituitary, Pancreas, Adrenal and Thyroid in Dogs and Rabbits. Oliver Cope, Israel Kapnick, Adrian Lambert, T. Dennie Pratt and Max G. Verlot, *Boston, Massachusetts.*

Attempts to Influence the Growth and Metastasis of the Brown-Pearce Epithelioma in Rabbits with Insulin. John R. Murlin, C. D. Kochakian, T. B. Steinhausen and R. Ryer, *Rochester, New York.*

Influence of Insulin on Experimental Neoplasms. Milton O. Lee, William Freeman, R. G. Hoskins and Helen M. Levene, *Boston, Massachusetts.*

The Relief of Myxedema with Proteins of Extrathyroidal Origin. J. Lerman and W. T. Salter, *Boston, Massachusetts.*

A Comparative Study of the Development of the Clinical, Electroencephalographic and Biochemical Changes During Insulin Hypoglycemia. H. E. Himwich, J. P. Frostig and J. F. Fazekas, *Wingdale, New York.*

Fat Boys. Julius Bauer, *New Orleans, Louisiana.*

Induction of Human Breast Growth by Percutaneous and Intramuscular Administration of Ovarian Hormones. Cyril M. MacBryde, *St. Louis, Missouri.*

The Effect of Prolactin on Mammary Gland Secretion. H. L. Stewart, Jr. and J. P. Pratt, *Detroit, Michigan.*

The Diagnostic Significance of Cranial Roentgenograms in Pituitary Disease. Max Goldzieher, *New York, New York.*

5:00 P.M.

ANNUAL BUSINESS MEETING

PAPERS TO BE READ BY TITLE

Clinical Experience with Antuitrin (Growth) in Two Diabetic Pituitary Dwarfs (Lorain-Levi). L. J. Bailey and S. S. Altshuler, *Detroit, Michigan.*

Simmonds' Disease Following Miliary Tuberculosis: Response to Treatment. Harvey G. Beck and George M. Suter, *Baltimore, Maryland.*

Studies on the Excretion of Pregnandiol in Cases of Sterility. J. S. L. Browne, Eleanor H. Venning, J. S. Henry and C. J. Pattee, *Montreal, Canada.*

The Occurrence of Urinary Calculi in an Inbred Strain (C_3H) of Mice Treated with Estrogen. Edward L. Burnes and John R. Schenken, *New Orleans, Louisiana.*

The Therapeutic Use of Pregnancy Urine by Mouth. Louis Cohen, *St. Louis, Missouri.*

The Endocrine Aspect of Juvenile Delinquency. J. K. Fancher, *Atlanta, Georgia.*

Indications for Androgen Therapy in Gynecology. Samuel H. Geist, Udall J. Salmon, Joseph A. Gaines and Robert Walter, *New York, New York.*

A Case of So-called Albright's Syndrome. Robert C. Grauer, C. W. W. Elkin and Paul B. Steele, *Pittsburgh, Pennsylvania.*

Gonadotropic Hormone-Assays of Human Male Urine. Emily J. Heller, Carl G. Heller and Elmer L. Sevringhaus, *Madison, Wisconsin.*

Metabolism of the Estrogens:—in Vitro Studies of the Effect of Uterine Tissue. Carl G. Heller, Emily J. Heller and Elmer L. Sevringhaus, *Madison, Wisconsin.*

The Assay of Thyrotropic Activity by the Increases of Thyroid Weights in One-Day-Old Chicks. LEONARD R. HINES, JOHN S. EVANS, JOSEPH J. CEITHAML AND F. C. KOCH, *Chicago, Illinois.*

Obesity in Children from the Endocrine Standpoint. LOUIS A. LURIE, *Cincinnati, Ohio.*

The Estrin Content of the Follicular Fluid and Urine of the Mare and its Relation to the Psychological and Physiological Phenomena of the Estrual Cycle. DENNIS T. MAYER, FREDERICK N. ANDREWS AND FRED F. MCKENZIE, *Miles City, Montana.*

Masculinizing and Non-Masculinizing Carcinomata of the Cortex of the Adrenal Gland. THOMAS H. MCGAVACK, *New York, New York.*

The Effect of Anterior Pituitary-Like Sex Hormone on the Blood Picture in Man. WILLIAM A. MOFFAT, *Santa Barbara, California.*

Observations on Unsettled Problems of Graves' Diseases in Childhood. WILLIAM A. REILLY, *San Francisco, California.*

Carbohydrate, Fat and Protein Appetite of Pancreatectomized Rats. CURT, P. RICHTER AND EDWARD C. H. SCHMIDT, JR., *Baltimore, Maryland.*

Some Quantitative Aspects of the Placentomata Reaction in the Rat. IRVING ROTHCHILD, ROLAND K. MEYER AND M. A. SPIELMAN, *Madison, Wisconsin.*

An Unusual Case of Deficiency Disease in a Patient with Diabetes Mellitus. ABRAHAM RUDY, *Boston, Massachusetts.*

The Effects of Feeding High Fats Diets Containing Varying Amounts of Protein to Hypophysectomized Animals. LEO T. SAMUELS, ROGER M. REINECKE, HOWARD A. BALL AND ROBER COHEN, *San Diego, California.*

The Non-Endocrine Maintenance of Adrenalectomized Rats. ARTHUR STEINBERG, *Philadelphia, Pennsylvania.*

A New Relation of the Thymus to the Adrenal Gland. ARTHUR STEINBERG, *Philadelphia Pennsylvania.*

Studies on The Effect of Pellets of Desoxy-Corticosterone Acetate Implanted Subcutaneously. GEORGE W. THORN, LEWIS L. ENGEL AND KENDALL EMERSON, JR., *Baltimore, Maryland.*

Clinical Significance of Glycosuria. B. A. WATSON, *Minneapolis, Minnesota.*

Papers read at the Annual Meeting are to be submitted to the editor for publication in *Endocrinology*. A copy of each paper should be handed to the secretary at the time of reading.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright	Murray B. Gordon	A. E. Meyer
Wayne J. Atwell	Arthur Grollman	Warren O. Nelson
Geoffrey Bourne	Frank A. Hartman	J. P. Pratt
Israel Bram	E. C. Hamblen	A. T. Rasmussen
John C. Burch	F. C. Koch	Elmer L. Sevringhaus
H. J. Duel, Jr.	Edward Larson	L. B. Shpiner
John C. Donaldson	J. T. Lewis	D. L. Thomson
Maurice H. Friedman	Joseph M. Looney	R. L. Zwemer

ADVANCE ABSTRACTS

BISCHOFF, H. W., AND W. R. LYONS. Immunologic investigation of hypophyseal mammotropic preparations. *To appear in this Journal.*

Mammotropic (lactogenic) preparations made from beef or sheep pituitaries were found to be antigenically indistinguishable as far as could be determined by the technics employed for active and passive anaphylaxis, Dale and Arthus reactions, precipitin and complement fixation tests. As little as 12 micrograms of beef or sheep mammotropin sufficed to produce anaphylaxis in sensitized guinea pigs, whereas normal guinea pigs receiving 20 milligrams showed no bad effects. Severe skin reactions were produced in rabbits sensitized to either beef or sheep mammotropin when they were injected intradermally with 1.0 mg. of either beef or sheep mammotropin. The mammotropic preparations were free from serum proteins and whether made from beef or sheep pituitaries they produced in rabbits, precipitins or complement fixing antibodies capable of reacting equally well to like concentrations of either sheep or beef mammotropin. Antisera capable of detecting 1 microgram or less of either beef or sheep mammotropin, by precipitin test, were able to neutralize the crop-stimulating effect of both beef and sheep mammotropin.

BRACK, C. B., AND O. R. LANGWORTHY. The effect of estrogenic hormone on vesical muscle. *To appear in this Journal.*

Twenty observations were made of the bladder capacity of 11 castrated cats following the administration of progynon-B. In 15 instances, or 75%, there was a significant decrease in bladder capacity. In 4 instances, or 20%, there was no demonstrable change in capacity, and in only one instance was there an increase in vesical capacity. These results suggest that there is a relationship between the presence of the female sex hormone and the vesical muscle tone, and that in the presence of the estrogenic hormone the tone is increased.

DORMAN, R. I., AND J. B. HAMILTON. The urinary excretion of estrogenic substances after the administration of testosterone propionate. *To appear in this Journal.*

After administration of the "testis" male hormone, testosterone propionate, to 2 men with deficient testicular secretion and to 4 immature *Macacus rhesus* monkeys (average body weight 2 kg.), the urine was examined with regard to change in estrogenic activity. Since all assays were made on the alkali-soluble fraction of the urine extract, the estrogenic substances determined were phenolic, hence chemically related, if not known naturally-occurring estrogens like estrone or estriol.

DORFMAN, R. I., AND J. B. HAMILTON. The urinary excretion of androgenic substance after the administration of testosterone propionate to the immature monkey. *To appear in this Journal.*

The quantitative excretion of androgenic substances in the urine after the intramuscular administration of testosterone propionate has been investigated in immature male and female monkeys. If the percentage of recovered androgens is calculated on the basis that testosterone is converted to and excreted as androsterone, the administration of 20 mg. of hormone in a single dose results in the recovery of between 1.2 and 6.4%. After a single dose of 5 mg. between <1.4 and 1.7% is excreted. When 340 mg. in divided doses was administered on alternate days for over a month, between 5.4 and 15.9% was found in the urine. After the injection of 70 and 85 mg. in 30 days, 7.8 and 11%, respectively, were

present in the urine. If the urinary androgenic substances were testosterone the percentage recovered would be one-sixth of the above figures, if dehydroisoandrosterone 3 times greater. With continuation of the administration of testosterone, the amount of androgenic substance in the urine is increased over that present during the first days of injection.

ENGEL, P. Assay of pineal extracts. *To appear in this Journal.*

The results of Fischer are confirmed. The inhibition of the opening of vaginal membranes of infantile mice is considered the best test for pineal extracts. The difference in the results of different investigators are caused by the existence of two hormones in the pineal gland.

ENGEL, P., AND C. E. MORALES. The Friedman reaction in infantile female rabbits. *To appear in this Journal.*

The Friedman reaction in female rabbits of 700 to 800 gm. weight remains negative in Europe, whilst it gives positive results in a tropical or sub-tropical climate.

FEVOLD, H. L., AND V. M. FISKE. The inhibition of the action of the follicle stimulating hormone by the pituitary. *To appear in this Journal.*

Estrogen or luteinizing hormone preparations inhibit the action of FSH on the ovaries of immature rats when injected for 8 days previous to FSH treatment. In hypophysectomized immature rats, estrogen does not produce this inhibition whereas the LH preparations are as active as in normal rats. Estrogen therefore produces the effect by stimulating the pituitary to secrete the same substance which is administered to the rats in the LH preparations. The inhibiting substance does not produce inhibition by direct action on the ovaries, making them refractory to FSH, since the ovaries from rats which had been treated with estrogen for 8 days responded to FSH when implanted into animals which had not been treated with estrogen. On the other hand, ovaries from normal rats implanted into treated rats did not respond, showing that the bodily environment determined the responsiveness of the ovaries. Assay of the blood of rats treated with estrogen for 8 days showed that LH was present in greater concentration than in normal rats or in rats which had received estrogen for only 3 days. Correlated with this fact is the augmentation of the FSH action after 3 days and inhibition of FSH action after 8 days of estrogen treatment. Attempts to separate the antagonistic principle from the luteinizing hormone were unsuccessful. It seems that the inhibiting agent either is the LH or a factor which is closely associated with and very similar to it.

HAMBLEN, E. C. The clinical evaluation of ovarian responses to gonadotropic therapy. *To appear in this Journal.*

This review points out the various difficulties inherent in attempts to evaluate clinically ovarian responses to gonadotropic therapy. Pertinent methods of clinical observation are described. The need of therapeutic tests for the gonadotropes is considered and the advisability of such to determine the receptivity of the ovaries is pointed out. A method for carrying out what would seem to be a satisfactory therapeutic test is described. Consideration of the clinical data available indicates that there exists little definite proof that therapeutic efficiency has been established for the gonadotropes in most supposed endocrinopathic gynecology. Augmentation of normally occurring ovarian responses may be produced by injections of gonadotropes. The existence of varying degrees of ovarian and endometrial refractivity or lowered receptivity may be responsible for failure of desired therapeutic results in functional bleeding and in ovarian sterility, rather than lack of specificity of the gonadotropic extracts. Patients with symptoms and signs of primary or early hypoovarianism seem most likely to be benefitted by this therapy. These should be given an adequate trial of gonadotropic treatment before replacement therapy with the ovarian sterols is initiated.

HAMBLEN, E. C., W. K. CUYLER, N. B. POWELL, C. ASHLEY AND M. BAPTIST. Some clinical observations upon the metabolism and utilization of crystalline progesterone. *To appear in this Journal.*

Endometriotropic responses were studied during 99 of 117 cycles of therapy with estrogens and/or progesterone given 23 women. Some degree of progestational alteration was encountered at the end of 22 cycles, but only one normal progestational endometrium occurred, the remainder being classified as mixed. Urinary titers of sodium pregnandiol glucuronide were investigated during 40 cycles of 7 women, who received similar therapy during 23 of these cycles. No evidence was encountered in these studies that crystalline progesterone, when given intramuscularly in oily solution is metabolized into sodium pregnandiol glucuronide. Apparently, when given alone, progesterone depresses the spontaneous metabolism of endogenous progestin. Endometriotropic studies suggest the possibility that the concurrent administration of estrogens with progesterone may enhance the utilization of the latter. The ineffective utilization of crystalline progesterone by the endometria of these patients seems related to an inadequate metabolism of that sterol.

Hoskins, R. G., H. M. Levene and S. Bevin. The relationship of male sex hormone to the level bodily vigor in senility. *To appear in this Journal.*

In 16 experiments on 9 senescent or senile male rats injections of testosterone propionate failed to result in any significant augmentation of muscular activity as shown in self-registering activity cages. It is concluded that the bodily inactivity of the elderly is not due to lack of testosterone.

Jensen, H., M. E. Simpson, S. Tolksdorf and H. M. Evans. Chemical fractionation of the gonadotropic factors present in sheep pituitary. *To appear in this Journal.*

A method is described for the separation of 2 gonadotropic fractions from sheep pituitary, a) FSH, M.E.D. 0.025 mg. in hypophysectomized female rats, using subcutaneous and intraperitoneal methods of injection, and b) ICSH, M.E.D. 0.0125 mg. in hypophysectomized animals upon intraperitoneal injection. Either fraction is free of the other up to 10 to 20 times the M.E.D. The separation and purification of the two fractions described increases the potency about a hundred-fold over that characterizing the initial dried sheep pituitary powder. A brief description of the physiological behavior of each fraction, and of the combination of the two, is given, showing that such physiological effects as synergism (activation), luteinization and antagonism can be secured by simultaneous administration of the two fractions designated as FSH and ICSH.

Lauson, H. D., J. B. Golden and E. L. Sevringhaus. The rate of increase in hypophyseal gonadotropic content following ovariectomy in the rat, with observations on gland weights. *To appear in this Journal.*

The rate of increase in pituitary gonadotropic potency following ovariectomy in the rat has been investigated. The gonad-stimulating content of the hypophyses from normal 24-week-old females and females of the same age gonadectomized 5, 10, 20, 30, 60 and 120 days prior to autopsy was evaluated in terms of an arbitrary uterine unit. The potency per gland in the respective castrated groups was found to be 3.7, 7.0, 20.7, 31.2, 52.7 and 51.9 times greater than normal. Body weight and length increased in proportion to the time elapsed between castration and autopsy. There was no consistent effection on pituitary and thyroid weights, and no significant atrophy of the adrenals was found. Hypertrophy of the thymus reached a maximum increase of 240% over normal by the 30th day after ovariectomy. Increase in kidney weight was roughly proportional to body growth except in the 60 and 120-day castrates.

Link Koven, A., and H. H. Beard. Creatine-creatinine metabolism and the hormones. II Progestin and antuitrin-T. *To appear in this Journal.*

Commercial progestin, crystalline progesterone and antuitrin-T were injected into young rats before and after castration to study their effects upon creatine-creatinine metabolism. Castration leads to a temporary creatinuria, but does not affect the concentration of tissue creatine in the rat. Likewise the injection of progestin or antuitrin-T does not change the concentration of muscle creatine. These hormones give a more intense creatinuria in the castrate as compared to the normal animal. Progestin causes an initial increase followed by a decrease in creatinine excretion in normal animals which is accompanied by an increase in creatine excretion. Antuitrin-T causes an increase in the excretion of both creatine and creatinine in the normal animal. A possible explanation is given of the effect of the sex hormones on creatine-creatinine metabolism.

Moehlig, R. C. Acromegaly. *To appear in this Journal.*

A case of acromegaly, seen from its inception until death, 8 years later, is reported. Polyuria and polydipsia were a clinical feature and the possibilities of its causes are discussed. Noteworthy besides the usual findings of splanchnomegaly, was the enormously enlarged heart, a hemorrhagic cyst in an eosinophilic and basophilic pituitary gland, hypertrophy and hyperplasia with solid adenoma formation of the parathyroid glands, marked enlargement with hypertrophy and hyperplasia of the right adrenal cortex, unilateral (right) hydronephrosis and hydroureter with the presence of a ureteral stone. Roentgen therapy had been given to the pituitary region. Death was the result of myocardial failure.

Myerson, A., and R. Neustadt. Influence of ultraviolet irradiation upon excretion of sex hormones in the male. *To be published in this Journal.*

Ultraviolet irradiation of man, applied with a small mercury quartz lamp, increases the excretion of androsterone in urine. Measurements were made colorimetrically. Irradiation of the scrotum and its vicinity proves to be more effective than irradiation of other parts of the skin. The experiments furnish no proof that the pituitary plays a primary rôle in the stimulation of increased androgenic activity. The possibility that a seasonal factor influences the result of the experiments is discussed.

Ross, D. A., and R. S. Schwab. The cortical alpha rhythm in thyroid disorders. *To appear in this Journal.*

A group of 42 patients with thyroid dysfunctions including myxedema and hyperthyroidism were examined by means of the electroencephalograph. The percentage of alpha rhythm and the number of alpha waves per second were measured in this group of patients and charts were made to show the relationship of these waves to the B.M.R. In the whole group of 80 determinations a coefficient of correlation was found to be .688. It was found that the lower the B.M.R. the slower the alpha waves. For example; a patient with a B.M.R. of −40 showed alpha waves of 6.5 per second, whereas the normal is 10 per second, and in patients with a B.M.R. of +30 would show a rhythm of 12 to 14. In an effort to increase the accuracy of the correlation the brain waves were done in a small number of patients in the same basal conditions required for the B.M.R. In this group the coefficient of correlation was less than in the whole group (.4), and in the non-basal group where the electrical tests were done any time during the day the coefficient of correlation was .883. This work suggests that the cortical alpha rhythm is related to the general metabolic rate and represents cortical metabolism in the same way.

Rubinstein, H. S., A. R. Abarbanel and A. A. Kurland. The effect of castration on body weight and length of the male albino rat. *To appear in this Journal.*

Castration of immature white male rats (22 days of age) leads to a suppression of somatic growth as determined by body weight and body length, both of which are significantly inhibited. Body length, however, is more affected than body weight so that by 80 days of age the animal has become relatively heavy for its body length.

Sutton, T. S., and B. J. Brief. Physiological changes in the anterior hypophyses of vitamin A deficient rats. *To appear in this Journal.*

In continuation of our studies of the effects of vitamin A deficiency on the pituitary gland, (*Endocrinology* 23: 211. 1938) anterior pituitaries from vitamin A deficient rats were assayed for gonadotropic hormone. In the assay technic employed the degree of gonad stimulation was measured by the changes in the vaginal epithelium of the assay animals (immature female mice). The sensitivity of the technic is illustrated by the fact that the administration of 0.14 mg. of pituitary substance gave a measurable stimulation. The data obtained show that pituitary substance from normal males has greater gonadotropic activity than that from normal females. In vitamin A deficiency the gonadotropic hormone content of the pituitary is increased. This increase in potency is greater in the male than in the female.

Swann, H. G. Some experiments on salt and water metabolism in diabetes insipidus. *To appear in this Journal.*

In some aspects of diabetes insipidus in the rat, the polyuria appears primary while in others the polydipsia appears primary. Thus, total nephrectomy prevents the polydipsia of both the transient and the permanent phases of diabetes insipidus, providing the animals are drinking water. If, however, they are drinking saline and if they have come to the permanent phase of diabetes insipidus, the nephrectomy does not abolish the polydipsia. The animals consequently ingest a large amount of fluid that they cannot excrete; this is partially stored in the skin, a massive edema of the subcutaneous connective tissue being caused. The case of one rat is reported in which the polydipsia was clearly primary for the animal ingested saline at a rate faster than it could excrete it, viz. from 2000 to 2500 cc. per kg. per day. The observation that animals with diabetes insipidus can tolerate fluid restriction fairly well has been confirmed; even after extreme aggravation of the condition with salt, fluid restriction for long periods is not harmful. The observation that in diabetes insipidus the animal can be forced to secrete a urine concentrated in NaCl has also been confirmed.

Thompson, W. O., P. K. Thompson, S. G. Taylor, III, and W. S. Hoffman. The treatment of Addison's disease with adrenal cortex. *To appear in this Journal.*

Observations with an adrenal cortex extract in the treatment of Addison's disease show that from 10 to 20 cc. daily will often maintain life for long periods without any other form of therapy, but larger amounts are probably necessary to produce optimum conditions. In a crisis, it is important to administer intravenously per hour, 10 cc. of an active adrenal cortex extract and about 170 cc. of 5% dextrose in normal salt solution, until vomiting stops and appetite returns. There is no danger at present of giving and overdose of extract. Early in a crisis a patient may be revived by extract alone. When a crisis is well advanced extract alone is not effective, presumably because of depletion of the sodium reserves of the body. Following recovery from a crisis, patients may be maintained: *a*), by administration of an active adrenal cortex extract in an adequate (at least 10 cc. daily), *b*), by administration of about 12 gm. of NaCl and 4 gm. of sodium citrate or bicarbonate daily by mouth (The combination works better than NaCl alone), *c*), by a combination of *a*) and *b*); *d*) the diet should always be high in calories and, according to

Wilder, Allers and Kendall, low in potassium. Failure to gain weight is usually a serious omen. An adequate dose of the extract appears to produce a more satisfactory clinical condition than sodium salts alone. If necessary, because of expense, the extract may sometimes be held in reserve for crises and patients otherwise maintained by sodium salts with or without a low potassium diet. The dose of the extract should be increased during an infection. If no active tuberculosis infection is present, patients may be maintained in a satisfactory condition for long periods. There appears to be some relationship between the nutritional state of the patient and the level of the blood pressure. When the basal metabolism is low, raising it to normal with a suitable dose of thyroid is an important part of the treatment. Under such circumstances we have not observed any aggravation of the disease from its use. There appears to be a relationship between the function of the ovary and the adrenal cortex.

A COMPOSITE ABSTRACT OF SOME ARTICLES ON THE ADRENALS NOT PREVIOUSLY ABSTRACTED IN THIS JOURNAL

ADRENALS

EIGHT CASES of female pseudohermaphroditism are reported (1). Hyperplasia of the inner layer of the adrenal cortex was present in all 6 in whom the adrenals were examined. The pelvis contained atrophic female sex organs and the clitoris was hypertrophied so that it was almost the size of a penis. The libido was definitely masculine.

Seven cases of adrenal cortical tumor are reported (2). Five occurred in women and two in girls. All showed different degrees of change in distribution of weight, distribution of hair, appearance of skin, menstruation and loss of strength. Some showed elevation of blood pressure, prolonged hyperglycemic reaction and decalcification of bone. The symptoms of adrenal cortical tumor in children and in adults of each sex are described. The differential diagnosis from pituitary basophilism and certain other tumors producing a similar syndrome is discussed.

One of the above cases was previously reported (3). The patient was a girl of 19 with amenorrhea, hirsutism, disproportionate enlargement of face, trunk and abdomen, with striations of the skin and weakness. Her symptoms regressed promptly on the removal of an adrenal cortical tumor. The remaining adrenal cortical tissue is atrophic in many of these cases and careful pre-operative and post-operative treatment, details of which are given, is necessary. With the use of the treatment described 7 consecutive cases have been operated upon with no fatalities.

Another of the cases included in (2) was previously reported (4). The patient was a child 23 months of age showing precocious development. At 18 months she was nearly as large as her sister of 5 years. The centers of ossification in wrists and femur corresponded to those of a child of 5 to 6 years. There was also evidence of precocious sexual development. A tumor was removed with excellent results.

An adrenal cortical tumor in a woman 28 years old is reported (5). She had amenorrhea, high blood pressure, increase of weight about the face, neck and chest, scattered areas of decalcification of the skull, facial hair and weakness. She died following operation. A detailed autopsy report including findings in other endocrines is given.

A report of an ovarian and adrenal tumor in the same patient is given (6). No definite diagnosis was made on the first ovarian tumor but the possibility of its being an arrhenoblastoma was considered. The patient's symptoms of virilism receded for a time after its removal. A second pelvic tumor necessitating complete hysterectomy was diagnosed as of wolffian origin. Genital symptoms returned after its removal and a shadow above the left kidney, believed to be an adrenal tumor, was seen by x-ray.

An adrenal cortical tumor occurring in a male with the symptoms of Cushing's syndrome is described (7). He showed a localized gain in weight about the neck, face and trunk, elevated blood pressure, retinitis and albuminuria. There were no changes in the genitals or in sexual desire. He died following operation and a complete autopsy report is given. The pituitary showed no adenoma.

Precocious puberty, muscular development and ossification of epiphyses is described

in a boy of 12 (8). Unusual development of the genitals was noted at 4 to 5 years. He had to be shaved occasionally at 7 to 8 years and showed unusual muscular development. He did not continue to grow as he should have after the 9th to 10th year. A detailed laboratory and x-ray report is given. No tumor could be detected by x-ray and the advisability of operation was being considered.

A benign cystic pigmented adenoma of the adrenal gland is described (9). The pigment was melanin and the tumor was believed to be of medullary origin. A detailed description of the differentiation of the body pigments by various stains is given.

The various methods of differentiating tumors about the upper pole of the kidney by clinical, x-ray and laboratory means are given (10). Tumors in this region are classified in 5 groups. Eleven cases of various types of tumor are described, including 2 carcinoma and 1 pheochromocytoma of the adrenal gland. No endocrine symptoms were reported in the 3 suprarenal cases.

The frequency of metastases to the adrenals of malignant tumors occurring elsewhere is reported (11). From the results of 4000 autopsies it was found that metastases to the adrenals occurred in nearly one-fourth of all organically metastasizing tumors.

Two cases of elevated blood pressure associated with adrenal tumor are reported (12). The first was a woman of 62 with cardiac decompensation and anasarca who developed increasing blood pressure. At autopsy hyperplasia and an adenoma of the adrenal cortex were found. The second case was a man of 56 with paroxysmal attacks of hypertension, nervousness, palpitation, dizziness and precordial pain who experienced relief of symptoms after removal of a pheochromocytoma of the adrenal medulla. A table giving 29 cases from the literature in which hypertension was associated with cortical hyperplasia or tumor is included.

A woman who had complained of attacks of precordial pain, tachycardia, nausea and weakness for more than 30 years is described (13). Her blood pressure was found to be elevated during the attacks. A very large cystic medullary tumor was removed at operation. The patient made a good recovery and was free from further attacks. The tumor was a mixed pheochromocytoma and ganglioneuroma, well encapsulated.

A medullary tumor of the adrenal producing hypertension without paroxysms is reported in a woman, 29 years of age (14). Her B.M.R. was +61%. Removal of the tumor resulted in a fall of blood pressure to the normal level and of the B.M.R. to +11%.

A medullary tumor in a man, aged 29 years, associated with paroxysmal attacks of weakness, headache and compression in the chest with elevation of blood pressure is described (15). Removal of the tumor which was a pheochromocytoma produced relief from the symptoms.

A case of adrenal sympathicoblastoma (neuroblastoma) in a male child of 2½ years of age is reported (16) with detailed clinical, hematologic and pathologic data. A table is given summarizing the information found in 179 cases from the literature. All died in a few months. Very widespread metastases occurred. A detailed description of the tumor cells is given. A second similar case in a female child 2¼ years of age is reported in a footnote.

A case of adrenal sympathicoblastoma in a female infant 16 months old is reported (17). The origin and occurrence of this type of tumor is discussed. In the patient described the tumor developed very rapidly, death occurring in less than a month after symptoms were first noticed. At autopsy the kidney and the pancreas were invaded by the tumor and there were metastases to the lymph nodes. A detailed description of the tumor cells is given.

A case of adenosarcoma and one of neuroblastoma are compared (18). The first occurred in a girl 6 years old who had not been well for 3 years. The tumor was inoperable and was found at autopsy to replace the adrenal and kidney with extension into the liver and metastases. The tumor cells are described. The neuroblastoma occurred in a girl 3½ years old whose condition was discovered because of acute abdominal symptoms. The tumor could not be removed but the child had improved at the time of writing following x-ray therapy.

The x-ray findings in 3 cases of neuroblastoma are presented (19). All three showed similar elevation of the periosteum of the femora. Other clinical and x-ray findings are described.

A case of lymphangiectasia of the adrenal is reported (20) in a man 65 years old. The

patient died of heart failure and the condition was discovered at autopsy. Rounded cystic areas were seen on the surface of the gland. Most of the cysts were very minute and microscopic examination showed them to be connected with the lymph channels in the walls of the veins. There was no evidence of malignancy.

Hemorrhagic destruction of the adrenal with rupture is described in an infant (21). Delivery was normal and at full term and the baby seemed in good condition. Four days later he was acutely ill with pallor, rapid, shallow respiration, occasional twitching, feeble heart sounds and increasing anemia. The blood coagulated normally. Death occurred the next day. At autopsy there were ragged tears in the right adrenal and the abdomen was full of blood. A classification of the causes of hemorrhage into the adrenal in the newborn is given.

Active testicular tissue was found next to the capsule of the adrenal gland during routine examination of a man dying of cancer of the lung (22). No report of a similar case was found in the literature. In man the anlagen of the two tissues are closely associated during the 6th and 7th fetal week.

The hypophysis and adrenals were examined in specimens from 20 anencephalic monsters (23). All three parts of the hypophysis were found in only three of the specimens. The anterior lobe was present in all but varied in size and shape. The pars intermedia was identified in 6 specimens and the pars nervosa in 5. No definite relationship to the amount of brain tissue present was found. The adrenal glands were smaller than normal and the cortex appeared more like that seen in the adult. Evidence is presented that the adrenals were originally larger but had undergone secondary atrophy perhaps as a result of malfunction of the hypophysis. The other glands of internal secretion did not show any characteristic changes.

A study of adrenal cortical cells to determine their origin and development is reported (24). The methods used in preparing and staining the material are given. The adrenals were obtained from a number of different kinds of animals. Evidence is presented that the cells of the glomerular layer are derived from the capsule and that these gradually progress toward the medulla, assuming changes in structure as they go, until they reach the reticular layer, the inner cells of which are gradually destroyed. The structure and staining reactions of the cells in the different layers are given in detail.

The presence of hypertrophy of the inner layer of the adrenal cortex (the x-zone) in young female mice is described (25). The layer degenerated gradually with age and rapidly during the first pregnancy. Castration in males caused the development of an x-zone which gradually degenerated with age. Injection of male hormones prevented the development of this zone in castrated males. No x-zone occurred in pituitary dwarf mice, suggesting that it is dependent upon pituitary secretion.

No vitamin C was found in the medulla of the adrenals of the mouse by the acid silver nitrate test nor in the glomerular layer of the cortex (26). The two inner zones of the cortex showed a positive reaction. The x-zone stained deeply, suggesting that it is cortical tissue.

An inner cortical layer termed the 'juvenile cortex' is seen in rats of both sexes at about 3 weeks of age (27). This resembled the minimum development of the x-zone in mice. In the rat this layer disappeared in 2 to 3 weeks. Castration did not affect it but the maximum development of the prostate and of the juvenile cortex occurred at the same time in rats castrated at birth.

Hypophysectomy caused a progressive shrinkage of the Golgi apparatus of the adrenal cortical cells in rats (28). If adrenocorticotropic hormone was given to hypophysectomized rats beginning soon after operation hypertrophy rather than shrinking of the Golgi apparatus occurred. More lipoid was seen in the cells when the Golgi apparatus was hypertrophied.

Pieces of the adrenal gland of newborn rats were grown in vitro and transplanted to adult adrenalectomized rats (29). Ten of 20 such grafts grew and became functioning. Growing grafts in a medium containing the recipient's serum did not definitely increase their chances of survival. No grafts transplanted to normal animals survived.

Adrenal cortical extract in large doses over a period of 7 to 28 days produced very marked atrophy of the adrenal cortical cells (30). The doses used were very much above the physiological dose. The atrophy did not affect the medulla. The compounds A and B isolated from the adrenal cortex produced similar results. When adrenotropic extract of the anterior pituitary was given at the same time no atrophy occurred.

When a sufficient amount of adrenotropic hormone was given to prevent the atrophy which normally follows hypophysectomy large amounts of adrenal cortical hormone produced no atrophy in hypophysectomized rats (31).

Female rats showed less loss of general body weight, weight of the adrenals and thymus than males when treated with large amounts of adrenal cortical hormone (32). Whether this outcome is related to the greater amount of adrenal cortical tissue in females than in males is not known.

X-ray therapy of the adrenals in rabbits produced first a hyperemia and later marked degeneration of the fascicular and reticular layers (33). Degeneration was most marked at 3 months after treatment. The distribution of lipoid in the treated glands was very irregular.

Detailed electrocardiographic studies on dogs with adrenal insufficiency are reported (34). First an irregular heart beat occured, then bradycardia. The electrocardiograms showed numerous changes including extrasystoles absent P waves, changes in the T waves, various degrees of heart block and others described. When insufficiency was pronounced section of the vagus or atropinization produced no acceleration of the heart rate. A high salt diet prevented the appearance of these symptoms. The coronary arteries, heart muscle and gastric mucous membrane showed changes in dogs allowed to die of insufficiency.

Evidence is presented (35) that adrenalin acts on chemical receptors in the carotid sinuses producing afferent stimuli to the vasoconstrictor center, rendering it less reactive to incoming stimuli.

Hyperplasia of the glomerular layer of the adrenal cortex occurred in 5 dogs following denervation of both carotid sinuses (36). No continued arterial hypertension developed during the period of study, 4 to 80 days. No structural changes were seen in the parathyroids.

Continuous perfusion of dilute solutions of adrenalin in dogs produced an initial rise of blood pressure and then a gradual decline to the starting level in spite of continued perfusion (37). Increase or decrease of the amount of adrenalin or the rate of flow caused a second rise or a fall of blood pressure respectively. Previous injection of cocaine produced a more prolonged initial elevation of blood pressure during perfusion with the same amount of adrenalin or a second prolonged rise when injected after the blood pressure had returned to the normal level. It had no blood pressure raising effect when injected without adrenalin.

The effects of intravenous epinephrine on the blood pressure of normal people and those with essential hypertension are compared (38). A dilute solution of adrenalin was injected for 15 minutes. The rise of systolic pressure was about the same in both groups. There was less change in the diastolic pressure although patients with hypertension showed a decrease of diastolic pressure more frequently than did normal persons.

A colorimetric method of determining the amount of adrenalin in a solution by its conversion to a red-colored compound by an oxidase is described (39). The test was reliable in solutions up to 1:500,000. Methods of preparing the solution and performing the test are given in detail. Results obtained by this method and those on spinal dogs compared favorably.

A detailed study of the regulation of the melanophores in the lizard is reported (40). Hypophysectomy caused the lizards to turn light in color and remain so. Injection of extracts of the intermediate and posterior lobe of the pituitary caused most of them to darken. Control of color changes was shown to be through some substance in the blood, not the nerve supply. Injection of adrenalin in hypophysectomized lizards caused mottling. If the adrenals as well as the hypophysis were removed no mottling could be elicited but injection of adrenalin produced it.

When three groups of frogs were kept *a*), on an illuminated white background; *b*), on an illuminated black background; *c*), in darkness for 21 to 72 hours it was found that the adrenals of those kept in the dark contained the most adrenalin (41). Those on an illuminated white background contained the least, and those on an illuminated dark background an intermediate amount.

Various esters of desoxycorticosterone were prepared and tested on the adrenalectomized rat to determine the duration of their life sustaining effect (41). The palmate and the benzoate had the longest effect i.e., 25 and 20 days respectively from a single 10 mg. dose. Desoxycorticosterone acetate produced a progesterone effect on the rabbit uterus.

Injections of a solution of corticosterone or the addition of a small amount to the water in the tank caused a marked lengthening of the ovipositor in the bitterling (43).

The results of ligation of the lumboadrenal veins in cats and dogs with experimental diabetes are reported (44). The survival period of the cats was increased from 7 to an average of 20 days. Glycosuria and ketonuria were much reduced and the blood sugar and fat values were much nearer to normal than in other completely pancreatectomized animals. Two cats were sugar free when fasting. The D:N ration and the respiratory quotient were also closer to normal values.

The effect of denervation of the liver on O_2 consumption following the injection of epinephrine was studied (45). In control animals the injection of adrenalin increased the excretion of carbonic acid and the absorption of oxygen about 17%. No increased O_2 consumption was found in the operated animals following injection of adrenalin. Their gas metabolism did not vary.

Changes in the mouse adrenals after treatment with an ultrafiltrate of blood from patients with hypertension showed that it contained an adrenocorticotropic substance (46).

The effects of adrenalectomy, decorticalization and injections of adrenalin upon the proportions of nitrogen and water in the tissues and the protein distribution, stability and solubility in the liver, muscles and brain of the rat are described (47).

In cats which had not received barbital derivatives, ether anesthesia for 30 minutes produced a definite decrease of the adrenin content of the adrenal glands (48). Divinyl oxid used for the same period of time did not have any significant effect.

In normal dogs the intravenous injection of a solution containing morphine caused a fall of blood pressure, increase of blood sugar, and marked increase of epinephrine in the blood of the suprarenal vein (49) When this same dose was repeated daily or every 1 to 2 days the dogs became tolerant and no change in epinephrine output, blood sugar or blood pressure occurred.

Atropine injected intravenously in non-anesthetized dogs produced an increase of epinephrine in the suprarenal vein and an increase of blood sugar (50). The D_{10} to L_3 spinal roots had been previously cut and allowed to degenerate in these dogs. Morphine and insulin both increased the output of epinephrine. Injecting atropine half an hour before intensified their action on epinephrine secretion.

When the circulation to the head of a dog was isolated from that of the rest of the body and the brain was perfused with blood which was hypoglycemic an increase of adrenalin was found in the blood from the suprarenal vein (51). Vagotomy did not effect this but section of the cervical spinal cord prevented it. Suddenly increasing the amount of glucose in the blood perfusing the brain caused a definite decrease in the amount of adrenalin in the adrenal vein. Previous ingestion of sodium barbital prevented the increase of adrenalin following the injection of insulin. This action was believed to be through paralysis of the thalamic centers.

An experiment to test the effect of the removal of the neutrophils by antineutrophilic serum on the lymphocytic reaction to various substances is described (52). Several substances which normally lower the lymphocytic count were tested and all produced a similar reaction in treated and untreated guinea pigs. The presence or absence of the neutrophils had no appreciable effect on the lymphocyte reaction.

Experiments on the effect of extracts of cat uterus on adrenalin are reported (53). No evidence was found that extracts from the pregnant or nonpregnant uterus altered the action of adrenalin, rather they prevented its oxidation. Extracts of the cat uterus itself lowered blood pressure.

The production of a cardio-inhibitory substance by the oxidation of adrenalin is described (54). Phenolases obtained from various sources were used. Oxidation by phenolases was more rapid in the cardiac tissue than in vitro. Very exact conditions are necessary for these changes to take place. Five stages in the complete oxidation of adrenalin are listed.

A report is made on the results of x-ray therapy of the pituitary and adrenals in hypertension (55). The author treated 25 cases and also reports cases from his correspondence and the literature. He states that symptomatic relief was obtained in more than 75% of the cases.

That intranasal application of pituitrin-S and adrephine in monkeys afforded some protection against intranasal induction of experimental poliomyelitis is reported (56). About two-thirds of the monkeys treated were resistant to intranasal infection and two-thirds of these showed immune bodies in their blood 2 to 3 weeks later. Adrephine used alone gave almost the same results but pituitrin-S alone gave no protection. When these two drugs were given hypodermically no immunity resulted.

The results of treating 70 cases of malaria with adrenalin are reported (57). Daily increasing doses of adrenalin were given intravenously. The reaction of the patient had to be watched and the treatment modified as necessary as the higher doses were approached. Quinine was used in most instances to control fever. Parasites disappeared from the blood, the spleen contracted under the costal margin in all but one case, and the patients gained weight and strength. The results did not seem to be affected by the stage of development or type of plasmodiums. The treatment seems to have increased the immunity of patients returning to malarious districts.

The treatment of 50 cases of childhood asthma with a crude extract of adrenal cortex is reported (58). A semiketogenic diet and special exercises were also used with special attention to individual needs. Of these, 84% showed improvement in their allergic symptoms at the end of a year and 90% improvement in their general condition.

In the course of treating 51 patients with adrenal cortical extract for other conditions, especially asthma and asthenia, it was found that 27 of them who were especially susceptible to colds all but one noted improvement of this symptom (59).

Large doses of adrenal cortical extract given intravenously daily for 6 days, in conjunction with a high salt diet, produced definite alleviation of symptoms in only 1 of 19 patients with asthma and related conditions (60).

The use of adrenalin with a toxin or antitoxin mixture is discussed (61). It is believed that by delaying the too rapid absorption of these agents it helps to prevent marked local or constitutional reactions. It may also prevent reactions when an attempt is made to desensitize serum-sensitive individuals. Experiments were carried out on guinea pigs to show that it did not alter the immunologic action of the toxin or antitoxin mixture.

The use of hypertonic sucrose and adrenalin intravenously in status asthmaticus is is reported (62). Six patients received 12 injections with immediate relief in all but two instances. Sucrose and adrenalin were more effective than sucrose alone or adrenalin with hypertonic glucose.

The differential diagnosis of acute pylorospasm from ruptured peptic ulcer by means of adrenalin is discussed (63). The author reports 5 cases in which acute pylorospasm with severe upper abdominal pain was relieved by small repeated doses of adrenalin, thus making a quick differential diagnosis from the more serious condition.

The pre-operative and post-operative use of adrenal cortical extract to prevent the development of shock is reported (64). A case of a woman with gas gangrene infection who was almost moribund and recovered following the use of cortical extract and one of a boy with general peritonitis suggested to the author the possibilities of this drug. Two small doses were given intramuscularly pre-operatively and at intervals of 6 to 12 hours post-operatively as needed. The patients were more comfortable and the amount of narcotic necessary was much reduced. Distension or paralysis of the intestine did not occur in these patients. Food was retained earlier and appetite was quickly restored.

Two cases believed to be acute fulminating meningococcus septicemia are reported (65). One was a boy 4 years old, the other a girl of 6. Both showed a diffuse macular eruption, dark red to purple in color and enlarged hemorrhagic adrenals at autopsy. Both children died in less than 24 hours after they were first seen by the physician. This syndrome is known as the Waterhouse-Friderichsen syndrome.

Another case of Waterhouse-Friderichsen syndrome in a boy of 3 is reported (66). A diffuse petechial rash was present and the adrenals were so distended with blood as to look almost black. The child was ill only about 9 hours.

A report is made (67) on the use of adrenal cortical extract and vitamin B in certain complications of insulin shock therapy. Certain cases were sensitive to very small doses of insulin. Because insulin makes increased demands on the adrenals these cases were given 2

to 3 cc. of cortin daily. A second group of cases were slow to awake at the end of treatment after tube feeding with glucose. Most of these showed a very low blood sugar and were given intravenous injections of glucose plus a preliminary treatment with adrenal cortical extract as the latter aids in the absorption of carbohydrates. A few of this second group of cases, however, had hyperglycemia and these were given betaxin as it has been shown to cause a rapid fall in blood sugar.

A comprehensive review article on the present knowledge of the adrenal glands and their function is presented by Harrop (68).

The occurrence of Addison's disease in children is described (69). Forty cases occurring in children under 14 years of age have been collected from the literature and are presented with a short history of each. Most of the cases occurred in children 10 to 14 years old. Only 5 were found in children under 10. In at least 25 of these cases the adrenals were tubercular.

A case is reported in which the symptoms of adrenal insufficiency resembled those of the Adams Stokes syndrome (70). A young man 27 years old, working in the tropics, had been complaining of easy fatigability, dizziness and headache for two months. Suddenly early in the morning he collapsed with a pulse of 30–35. He showed slight improvement following heart stimulants but about a week later he had another sudden collapse, this time with a pulse of 25–30, slight paralysis of one side of his face and difficulty in speaking. On admission to the hospital his blood pressure was 60/40. There was a marked increase in the period of diastole, no heart block. Small doses of adrenalin every 4 hours increased the pulse rate and blood pressure. Discontinuance of it caused recurrence of symptoms.

A case of amyloidosis of the adrenals causing Addison's disease in a man 30 years old is reported (71) It is stated that the adrenals are frequently included in a general amyloidosis but the degree of involvement is very rarely sufficient to cause Addison's disease. The patient had pulmonary tuberculosis but the adrenals did not show any tuberculous lesions.

A case of Addison's disease showing marked improvement with ascorbic acid is reported (72). The patient was 31 years old and showed weakness, pigmentation, attacks of vomiting and diarrhea and lack of sexual desire. Treatment with intravenous salt solution and adrenal cortical extract produced no improvement. Ascorbic acid in daily single 1 gm. doses produced improvement from the third day. His appetite returned and vomiting and diarrhea stopped. Ascorbic acid did not appear in the urine until the 5th day of treatment. By the 12th day the patient was up and about, ate well, his frigidity had disappeared and he wanted to go home. A sore throat developing at this time did not cause a recurrence of symptoms.

Two cases of Addison's disease are reported (73). A general discussion of the symptoms is given. The first patient was a male 36 years old and the second a male of 40. Both patients received a high salt diet and eschatin and in addition 6 to 18 ounces of orange juice daily. Withdrawal of the orange juice in both cases caused exacerbation of the symptoms. The first case was milder and recovered with treatment, the second case was more severe and the patient died about 5 months after admission.

Corticosterone, dehydro-corticosterone and desoxycorticosterone acetate were found to decrease the excretion of sodium and chloride and increase that of potassium in normal dogs (74). Three other compounds obtained from the adrenal cortex did not have this effect. The effectiveness of these drugs on electrolyte excretion and their life-prolonging potency in adrenalectomized dogs were found to run parallel.

In normal rats cortin did not cause a retention of sodium salts or more than a transient rise in the excretion of potassium (75). Because insulin is known to have a potassium-retaining effect partially pancreatectomized rats were tested. In these rats cortin caused a great loss of weight and excretion of potassium. Some of these rats showed glycosuria and some did not. The rate of excretion of potassium and glucose showed no definite relation. When 100 mg. of potassium chloride was given to one of the rats which had shown only a slight glycosuria a very great excretion of glucose resulted. Partially pancreatectomized rats on cortin drank an enormous amount of water with a corresponding excretion.

Injections of both male and female sex hormones in normal male dogs caused a lessened excretion of sodium and chloride and frequently a slight increase of potassium excretion (76). Similar results occurred in adrenalectomized dogs showing that the effect was not neces-

sarily through the adrenals. Testosterone propionate had a comparatively feeble effect. Excretion of inorganic phosphorus and total nitrogen was decreased by injections of all except progesterone. Progesterone alone had a slight effect in prolonging the life of a suprarenalectomized dog.

In 12 persons studied an intramuscular injection of 1 mg. of adrenalin caused a fall of serum potassium (77). In 3 patients whose urine was examined this was followed by a diminished excretion of potassium. No constant relation between the blood pressure, blood sugar and fall in potassium level was found in these experiments.

It was found that the amount of chlorides excreted on the morning of the third day of a standard low salt, high potassium diet was of more diagnostic value in adrenal insufficiency than any of the blood chemical studies or other findings in the urine (78). A standard procedure for this test is described.

Cats with diabetes insipidus died sooner following adrenalectomy than normal cats but no decrease in their serum sodium or chloride occurred (79). The serum potassium increased. The general appearance of the two groups of cats with insufficiency was the same.

—Dorothea Whitney

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Sazawa. N. Influence of atropine upon the reduction of epinephrine content of the suprarenal gland due to certain agencies. *Tohoku J. Exper. Med.* 34: 470. 1938.

Experiments were done on rabbits to determine the effect of atropine upon the reduction of epinephrine by splanchnic stimulation, by bleeding or by insulin. Twenty-two rabbits were injected subcutaneously with 45 to 85 mg. of atropine per kg. of body weight, after which the left splanchnic nerve was cut and stimulated electrically. The suprarenal glands were then removed. They were found to contain no more epinephrine than those of other rabbits who had received no atropine. Other rabbits, after having received 53 mg. of atropine per kg. of body weight, were bled from the right carotid. These animals likewise showed no increase in epinephrine when compared with controls. Still other rabbits received 65 to 226 mg. of atropine per kg. of body weight, after which they were injected with 3 or 5 u of insulin per kg. of body weight. Similar results were obtained with these animals. The author concluded from these experiments that atropine does not interfere with reduction of the storage of epinephrine in rabbits.—Margaret Baptist.

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The purpose of the experiment was to study the difference between intact and adrenalectomized dogs with respect to their susceptibility to identical shock-inducing procedures. For the work, 13 animals bilaterally adrenalectomized and maintained on adrenal cortical extract for 6 to 12 mo., were used. In 7 of these, shock was produced by intestinal stripping (30 min.) and in 6, by muscular trauma. These were compared with identically treated, intact animals previously reported. Blood pressure, pulse rate, red cell volume and count, serum Na, Cl and K, and hemoglobin were determined before operation and during shock. The shock and circulatory collapse induced by intestinal manipulation or trauma to the muscles in the experimental animals were not due to changes in serum Na, Cl or K and associated disturbances in distribution of body H_2O. Hemoconcentration, although usually present to some degree, was not an invariable accompaniment of shock and circulatory collapse in the type of animal used in these experiments. Hypertonic saline given intravenously to the dog in profound shock, raised the arterial pressure to normal levels. The effect was temporary and the animal soon lapsed into shock. Adrenal cortical hormone when administered in adequate amounts restored the collapsed circulation to normal. The marked pressor effect of the hormone was apparently due to its regulatory control of capillary tone.—K. A. B.